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(54) Title: SEQUENCES CHARACTERISTIC OF BLADDER CANCER

(57) Abstract: There is provided a method of diagnosing the presence of bladder cancer in a patient by analyzing a tissue sample from the patient for the presence of at least one expressed gene wherein the presence of the expressed gene is indicative of bladder cancer. Also provided by the present invention is a polynucleotide sequence whose expression is indicative of bladder cancer. A marker for bladder cancer is also provided. There are also provided methods of diagnosing bladder cancer by screening for the presence of at least one expressed gene wherein the presence of the expressed gene is indicative of bladder cancer. Methods of treating and regulating bladder cancer-associated pathologies by administering to a patient a therapeutically effective amount of a ribozyme, antisense oligonucleotide, or agonist against the nucleic acids sequences of the present invention are alos provided.

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Exhibit 6

SEQUENCES CHARACTERISTIC OF BLADDER CANCER

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to the identification of polynucleotide sequences that are differentially expressed in bladder cancer. More specifically, the present invention relates to the use of the sequences and gene products for diagnosis and as probes.

DESCRIPTION OF RELATED ART

Bladder cancer is the second most-common genitourinary cancer in the United States, with only prostate cancer being more frequently diagnosed. Bladder cancer accounts for approximately two percent of all malignant tumors and approximately seven percent of all urinary tract malignancies in U.S. men. Over 54,000 new cases were estimated to be diagnosed in the United States in 1998, with approximately 12,500 deaths predicted [American Cancer Society, 1998]. The prevalence of bladder cancer is higher in industrialized nations, perhaps reflecting increased exposure to environmental carcinogens. Men are three times more frequently affected than women. The disease usually occurs between 60-70 years of age and the age-adjusted bladder cancer rate in white men is almost twice that of black men. Most bladder cancers (over 90%) are carcinomas of the transitional epithelium of the bladder's mucosal lining (transitional cell carcinoma (TCC)). Although 90 percent of the cases are localized at diagnosis, up to 80 percent recur.

A number of etiological factors are associated with the development of bladder cancer, but in industrialized countries, cigarette smoking is the most significant. Specific chemicals have also been identified as causing bladder cancer, as have a number of occupational exposures to less well-defined specific agents. Treatment with cytostatic drugs, especially cyclophosphamide, is associated with increased risk of bladder cancer, as is treatment with radiotherapy for uterine cancer.

Bladder cancer is a potentially preventable disease, with a significant morbidity and mortality in many parts of the world.

Tumors are graded according to the degree of cellular abnormality, with the most atypical cells being designated as high-grade (i.e., G3 grade) tumors. The major prognostic factors in carcinoma of the bladder are the depth of invasion into the bladder wall and the degree of differentiation of the tumor. The higher the grade of the tumor at the diagnosis, the higher the incidence of death from the disease within two years.

The stage of development of the tumor is significant in estimating disease prognosis. Most superficial, non-invasive tumors being papillary tumors which do not invade the *lamina propria*, and are classified as non-invasive TCC, i.e., "Ta" tumors, may recur, but nearly 70% will not progress further. A tumor which does not invade the muscle but does enter the *lamina propria* presents in many cases a worse prognosis. Such tumors are classified as non-invasive TCC, i.e., T1 tumors.

Most superficial tumors are well differentiated and classified as G1 grade tumors. Patients in whom superficial tumors are less differentiated, large, multiple, or associated with carcinoma *in situ* in other areas of the bladder mucosa, (classified as G2-G3 tumors) are at greatest risk for recurrence and the development of invasive cancer. Invasive bladder tumors tend to spread rapidly to

the regional lymph nodes and then into adjacent structures. Overall, the five-year survival rate of TCC is 76 percent for whites and 55 percent for blacks.

One of the management problems is the fact that carcinoma of the bladder is frequently multifocal. The entire bladder epithelium and the lining of the entire urothelial cell tract can undergo malignant change. After apparently successful treatment of a bladder lesion, new tumors may occur at the same site (recurrence) or in other urothelial cells in the bladder. Approximately 30 percent of bladder carcinomas present as multiple lesions at the time of initial diagnosis.

The early diagnosis of bladder cancer is central to the effective treatment of TCC. Presently, the detection of bladder tumors relies on intravenous pyelogram or other contrast studies to rule out urothelial involvement in the kidneys or ureters, and invariably cystoscopy which remains the accepted standard for diagnosis of mucosal abnormalities. There are no presently reliable methods available to easily and specifically identify the presence of bladder cancer cells. A variety of new technologies and potential tumor markers are being studied in bladder cancer and some are being translated into clinical use.

It is important to realize that all available results of the diagnostic value of tumor markers do not allow firm clinical recommendations, but tests based on biomarkers will undoubtedly influence the management of bladder cancer in the near future. Several new markers have been already identified and even approved for use (e.g. bladder tumor antigen (BTA) markers, NMP22, FDP). However, their clinical use is limited [Grossman, 1998], due to sensitivity and specificity problems in conjunction with cystoscopic examination.

Furthermore, due to the high rate of disease recurrence, follow-up of TCC patients is obligatory. There is a need to eliminate the invasive cytoscopy method of diagnosis and of follow-up and replace it with a reliable and non-invasive method of diagnosis.

Approximately 70-80 percent of patients with newly diagnosed bladder cancer will present with superficial, non-invasive bladder tumors. Those who do are often curable. Tumor patients with deeply invasive disease can sometimes be cured by complete surgical removal of the bladder, irradiation, or a combination of modalities that include chemotherapy, however the five-year survival rate is less likely for such tumors. It is therefore of major importance to detect new tools that will aid in both the initial early diagnosis and in follow-up of non-invasive TCC tumors.

Adverse prognostic features associated with a greater risk of disease progression include the presence of multiple aneuploid cell lines, nuclear p53 overexpression, and expression of the Lewis-x blood group antigen [Hudson and Herr, 1995; Lacombe et al., 1996]. It has been postulated that p53 may be useful for predicting the level of aggression of the tumor and to identify patients who will not benefit from chemotherapy. However, only a very small, select group of patients with invasive disease may benefit from this approach [Ozen, 1998].

Several treatment methods (i.e., transurethral surgery, intravesical medications, and cystectomy) have been used in the management of patients with superficial tumors, and each method can be associated with five-year survival in 55-80 percent of patients treated [Hudson and Herr, 1995; Torti and Lum, 1984]. Invasive tumors that are confined to the bladder muscle on pathologic staging after radical cystectomy are associated with an approximately 75 percent, five-year progression-free rate of survival. Patients with more deeply invasive tumors (which are also usually less well differentiated) experience five-year survival rates of 20-40 percent following radical cystectomy. When the patient presents with a locally extensive tumor that invades pelvic viscera or with metastases to lymph nodes or distant sites, a five-year survival rate is uncommon, but considerable symptomatic palliation can still be achieved.

Surgery is the main treatment method. The extent of surgery is dependent on the pathological stage of the disease. Early disease is generally treated by intravesical chemotherapy and transurethral resection. Locally invasive disease can usually be managed only by radical cystectomy and urinary diversion. Definitive (curative) radiotherapy is generally reserved for bladder cancer patients who are not candidates for surgery. For superficial, low-grade disease, chemotherapy is applied intravesically (directly into the bladder) to concentrate the drug at the tumor site and eliminate any residual tumor mass after resection. Systemic chemotherapy may also be used to manage advanced bladder cancer; compete response rates of 30-50 percent have been reported. Single agent chemotherapy has demonstrated limited success.

However, even following surgery and resection of non-invasive TCC tumors, frequent follow-up is required (every 3 months) in both non-invasive and invasive cases.

It would therefore be useful to be able to identify early stage TCC in bladder cancer which has a significantly higher cure rate and generally does not require surgery. In addition, it would be useful to identify markers that can be employed for early diagnosis and follow-up of both non-invasive and invasive TCC, as an efficient and non-invasive alternative to cytoscopy.

SUMMARY OF THE INVENTION

According to the present invention, there is provided a method of diagnosing the presence of bladder cancer in a patient by analyzing a tissue sample from the patient for the presence of a least one expresses gene wherein the presence of the expressed gene is indicative of bladder cancer. Also provided by the present invention is a polynucleotide sequence whose expression is indicative of bladder cancer. A marker for bladder cancer is also provided. There are also provided methods of diagnosising bladder cancer by screening for the presenceof at least

one expressed gene wherein the presence of the expressed gene is indicative of bladder cancer. Methods of treating and regulating bladder cancer-associated pathologies by administering to a patient a therapeutically effective amount of a ribozyme, antisense oligonucleotide, or agonist against the nucleic acids sequences of the present invention are also provided.

DESCRIPTION OF THE INVENTION

According to the present invention, purified, isolated and cloned nucleic acid sequences associated with bladder cancer are provided. More specifically, the sequences of the present invention are set forth in Table I or II or have a complementary or allelic variation sequence thereto. The nucleic acid sequences of the complementary or allelic variation sequences are provided in Tables III and IV, respectively.

When referring to bladder cancer, both invasive and noninvasive forms are included. Bladder cancers can also be referred to as transitional cell carcinomas or "TCC".

The present invention further provides a method of diagnosing the presence of bladder cancer in a patient, including the steps of analyzing a tissue sample from the patient for the presence of at least one expressed gene (up-regulated) wherein the mRNA from the expressed gene hybridizes to at least one of the sequences in Tables I or II, with hybridization occurring under conditions sufficiently stringent to require at least 95% base pairing.

Further the present invention provides antibodies directed against the gene products of the sequences of the present invention. The antibodies can be either monoclonal, polyclonal or recombinant and be used in immunoassays as described in the Methods herein below.

By regulate or modulate or control is meant that the process is either induced or inhibited to the degree necessary to effect a change in the process and the associated disease state in the patient. Whether induction or inhibition is being contemplated is apparent from the process and disease being treated and is known to those skilled in the medical arts. The present invention identifies genes for gene therapy, diagnostics and therapeutics that have direct causal relationships between a disease and its related pathologies and up- or down-regulator (responder) genes. That is, the present invention is initiated by a physiological relationship between cause and effect.

The present invention identifies polynucleotide sequences named in Tables I and II, and set forth in Tables III and IV, respectively, that can be utilized diagnostically in bladder cancer. Sequences named in Table I were found to match sequences in data banks and were newly found in the present application to be upregulated in TCC. The sequences named in Table II are either genes with unknown protein product or of unknown genes. All the sequences named in both Tables I and II were found to be associated with TCC relative to normal bladder samples.

Where the sequences are partial sequences, they are markers or probes for genes that are regulated in bladder carcinoma. By "regulated" it is meant that the genes can be either upregulated or downregulated, depending upon the specific gene. In general these partial sequences are designated "Expressed Sequence Tags" (ESTs) and are markers for the genes actually expressed *in vivo* and are ascertained as described herein. Generally, ESTs comprise DNA sequences corresponding to a portion of nuclear encoded mRNA. The EST has a length that allows for PCR (polymerase chain reaction), use as a hybridization probe and is a unique designation for the gene with which it hybridizes (generally under conditions sufficiently stringent to require at least 95% base pairing).

For a detailed description and review of ESTs and their functional utility see WO 93/00353 which is incorporated in its entirety by reference. WO 93/00353 further describes how the EST sequences can be used to identify the transcribed genes. The Example herein also describes a method of identification.

The present invention also provides a method of diagnosing the presence of bladder cancer in a patient, by the expression of at least one expressed gene (up-regulated) identified by the sequences of the present invention set forth in Tables I and II. Methods of identification of hybridization results can include, but are not limited to, immunohistochemical staining of the tissue samples. Further for identification of the gene, *in situ* hybridization, Southern blotting, single strand conformational polymorphism, restriction endonuclease fingerprinting (REF), PCR amplification and DNA-chip analysis using nucleic acid sequence of the present invention as probes/primers can be used.

Further, according to the present invention, purified, isolated and cloned bladder cancer associated genes identified by the probes and/or sequences hybridizing under stringent conditions with 95% homology and set forth herein, or a complementary or allelic variation sequence and human homologs, as relevant, thereto, are disclosed.

The present invention further provides proteins encoded by the identified genes. The present invention further provides antibodies directed against these proteins. The present invention further provides transgenic animals and cell lines carrying at least one expressible gene identified by the present invention. The present invention further provides knock-out eukaryotic organisms in which at least one nucleic acid sequences as identified by the probes of the present invention and prepared as described in the Methods.

The present invention provides a method of identifying bladder cancer, and particularly early stage associated pathologies in a patient. The present invention

provides a treatment by administering to a patient a therapeutically effective amount of an antagonist of at least one protein as encoded by the nucleic acid sequences or sequences identified herein or by the probes of the present invention. Alternatively, the present invention provides a method of regulating bladder cancer-associated pathologies in a patient in need of such treatment by administering to a patient a therapeutically effective amount of at least one antisense oligonucleotide against the nucleic acid sequences or dominant negative peptide directed against the sequences or their proteins.

Furthermore, the present invention provides a method of preparation of a library using proprietary methods of library preparation as follows: the SDGI method, as described in US Patent Application USSN 09/538,709 of same under the assignee, filed 30 March, 2000, and incorporated herein by reference in its entirety, the Antisense method as described in US Provisional Patent Application SN 60/157,843 of same assignee, filed 6 October, 1999, and incorporated herein by reference in its entirety, and suppressive subtraction hybridization (SSH) (Diatchenko et al., 1996).

Negative dominant peptide refers to a partial cDNA sequence that encodes for a part of a protein, i.e. a peptide (see Herskowitz, 1987). This peptide can have a different function from the protein from which it was derived. It can interact with the full protein and inhibit its activity or it can interact with other proteins and inhibit their activity in response to the full protein. Negative dominant means that the peptide is able to overcome the natural proteins and fully inhibit their activity to give the cell a different characteristics like resistance or sensitization to killing. For therapeutic intervention either the peptide itself is delivered as the active ingredient of a pharmaceutical composition or the cDNA can be delivered to the cell utilizing the same methods as for antisense delivery.

The antagonist or regulating agent or active ingredient is dosed and delivered in a pharmaceutically acceptable carrier as described herein below. The term antagonist or antagonizing is used in its broadest sense. Antagonism can

include any mechanism or treatment which results in inhibition, inactivation, blocking or reduction in gene activity or gene product and for example preventing progression from non-invasive to invasive. It should be noted that the inhibition of a gene or gene product provides for an increase in a corresponding function that the gene or gene product was regulating. The antagonizing step can include blocking cellular receptors for the gene products and can include antisense treatment as discussed herein.

Many reviews have covered the main aspects of antisense (AS) technology and its enormous therapeutic potential (Wright and Anazodo, 1995). There are reviews on the chemical (Crooke, 1995; Uhlmann et al, 1990), cellular (Wagner, 1994) and therapeutic (Hanania, *et al*, 1995; Scanlon, *et al*, 1995; Gewirtz, 1993) aspects of this rapidly developing technology. Within a relatively short time, ample information has accumulated about the *in vitro* use of AS nucleotide sequences in cultured primary cells and cell lines as well as for *in vivo* administration of such nucleotide sequences for suppressing specific processes and changing body functions in a transient manner. Further, enough experience is now available *in vitro* and *in vivo* in animal models and human clinical trials to predict human efficacy.

Antisense intervention in the expression of specific genes can be achieved by the use of synthetic AS oligonucleotide sequences (for recent reports see Lefebvre-d'Hellencourt *et al*, 1995; Agrawal, 1996; Lev-Lehman *et al*, 1997). AS oligonucleotide sequences can be short sequences of DNA, typically 15-30 mer but can be as small as 7 mer (Wagner et al, 1996), designed to complement a target mRNA of interest and form an RNA:AS duplex. This duplex formation can prevent processing, splicing, transport or translation of the relevant mRNA. Moreover, certain AS nucleotide sequences can elicit cellular RNase H activity when hybridized with their target mRNA, resulting in mRNA degradation (Calabretta *et al*, 1996). In that case, RNase H cleaves the RNA component of the duplex and can release the AS to further hybridize with additional molecules of the

target RNA. An additional mode of action results from the interaction of AS with genomic DNA to form a triple helix which can be transcriptionally inactive.

The sequence target segment for the antisense oligonucleotide is selected such that the sequence exhibits suitable energy related characteristics important for oligonucleotide duplex formation with their complementary templates, and shows a low potential for self-dimerization or self-complementation [Anazodo et al., 1996]. For example, the computer program OLIGO (Primer Analysis Software, Version 3.4), can be used to determine antisense sequence melting temperature, free energy properties, and to estimate potential self-dimer formation and self-complimentary properties. The program allows the determination of a qualitative estimation of these two parameters (potential self-dimer formation and self-complimentary) and provides an indication of "no potential" or "some potential" or "essentially complete potential". Using this program target segments are generally selected that have estimates of no potential in these parameters. However, segments can be used that have "some potential" in one of the categories. A balance of the parameters is used in the selection as is known to those of skill in the art. Further, the oligonucleotides are also selected as needed so that analogue substitution do not substantially affect function, as is known to those of skill in the art.

Phosphorothioate antisense oligonucleotides do not normally show significant toxicity at concentrations that are effective, exhibit sufficient pharmacodynamic half-lives in animals (Agarwal *et al.*, 1996) and are nuclease resistant. Antisense induced loss-of-function phenotypes related with cellular development were shown for the glial fibrillary acidic protein (GFAP), for the establishment of tectal plate formation in chick (Galileo *et al.*, 1991) and for the N-myc protein which is responsible for the maintenance of cellular heterogeneity in neuroectodermal cultures (ephithelial vs. neuroblastic cells, which differ in their colony forming abilities, tumorigenicity and adherence) (Rosolen *et al.*, 1990; Whitesell *et al*, 1991). Antisense oligonucleotide inhibition of basic fibroblast growth factor (bFgF), having mitogenic and angiogenic properties, suppressed

80% of growth in glioma cells (Morrison, 1991) in a saturable and specific manner. Being hydrophobic, antisense oligonucleotides interact well with phospholipid membranes (Akhter *et al.*, 1991). Following their interaction with the cellular plasma membrane, they are actively (or passively) transported into living cells (Loke *et al.*, 1989), in a saturable mechanism predicted to involve specific receptors (Yakubov *et al.*, 1989).

Instead of an antisense sequences as discussed herein above, ribozymes can be utilized. This is particularly necessary in cases where antisense therapy is limited by stoichiometric considerations (Sarver et al., 1990, Gene Regulation and Aids, pp. 305-325). Ribozymes can then be used that target the same sequence. Ribozymes are RNA molecules that possess RNA catalytic ability (see Cech for review) that cleave a specific site in a target RNA. The number of RNA molecules that are cleaved by a ribozyme is greater than the number predicted by stochiochemistry. (Hampel and Tritz, 1989; Uhlenbeck, 1987).

Ribozymes catalyze the phosphodiester bond cleavage of RNA. Several ribozyme structural families have been identified including Group I introns, RNase P, the hepatitis delta virus ribozyme, hammerhead ribozymes and the hairpin ribozyme originally derived from the negative strand of the tobacco ringspot virus satellite RNA (sTRSV) (Sullivan, 1994; U.S. Patent No. 5,225,347, columns 4-5). The latter two families are derived from viroids and virusoids, in which the ribozyme is believed to separate monomers from oligomers created during rolling circle replication (Symons, 1989 and 1992). Hammerhead and hairpin ribozyme motifs are most commonly adapted for trans-cleavage of mRNAs for gene therapy (Sullivan, 1994). The ribozyme type utilized in the present invention is selected as is known by those of skill in the art. Hairpin ribozymes are now in clinical trial and are the preferred type. In general, the ribozyme is from 30-100 nucleotides in length.

Modifications or analogues of nucleotides can be introduced to improve the therapeutic properties of the nucleotides. Improved properties include increased

nuclease resistance and/or increased ability to permeate cell membranes.

Nuclease resistance, where needed, is provided by any method known in the art that does not interfere with biological activity of the antisense oligodeoxy-nucleotides, cDNA and/or ribozymes as needed for the method of use and delivery (Iyer et al., 1990; Eckstein, 1985; Spitzer and Eckstein, 1988; Woolf et al., 1990; Shaw et al., 1991). Modifications that can be made to oligonucleotides in order to enhance nuclease resistance include, but are not limited to, modifying the phophorous or oxygen heteroatom in the phosphate backbone. These modifications also include preparing methyl phosphonates, phosphorothioates, phosphorodithioates and morpholino oligomers.

In one embodiment, the modification is provided by having phosphorothioate bonds linking between the four to six 3'-terminus nucleotide bases. Alternatively, phosphorothioate bonds can link all the nucleotide bases. Other modifications known in the art can be used where the biological activity is retained, but the stability to nucleases is substantially increased.

The present invention also includes all analogues of, or modifications to, an oligonucleotide of the invention that does not substantially affect the function of the oligonucleotide. The nucleotides can be selected from naturally occurring or synthetic modified bases. Naturally occurring bases include adenine, guanine, cytosine, thymine and uracil. Modified bases of the oligonucleotides include xanthine, hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza cytosine and 6-aza thymine, psuedo uracil, 4-thiuracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8-amino guanine, 8-thiol guanine, 8-thioalkyl guanines, 8-hydroxyl guanine and other substituted guanines, other aza and deaza adenines, other aza and deaza guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

In addition, analogues of nucleotides and/or polynucleotides can be prepared wherein the structure of the nucleotide and/or polynucleotide is fundamentally altered and that are better suited as therapeutic or experimental reagents. An example of a nucleotide analogue is a peptide nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in DNA (or RNA) is replaced with a polyamide backbone which is similar to that found in peptides. PNA analogues have been shown to be resistant to degradation by enzymes and to have extended lives *in vivo* and *in vitro*. Further, PNAs have been shown to bind stronger to a complementary DNA sequence than a DNA molecule. This observation is attributed to the lack of charge repulsion between the PNA strand and the DNA strand. Other modifications that can be made to oligonucleotides include polymer backbones, cyclic backbones, or acyclic backbones.

The active ingredients of pharmaceutical compositions can include oligonucleotides that are nuclease resistant as are needed for the practice of the invention or a fragment thereof shown to have the same effect when targeted against the appropriate sequence(s) and/or ribozymes. Combinations of active ingredients as disclosed in the present invention can be used, including combinations of antisense sequences.

The antisense oligonucleotides (and/or ribozymes) and cDNA of the present invention can be synthesized by any method known in the art for ribonucleic or deoxyribonucleic nucleotides. For example, an Applied Biosystems 380B DNA synthesizer can be used. When fragments are used, two or more such sequences can be synthesized and linked together for use in the present invention.

The nucleotide sequences of the present invention can be delivered either directly or with viral or non-viral vectors. When delivered directly the sequences are generally rendered nuclease resistant. Alternatively the sequences can be incorporated into expression cassettes or constructs such that the sequence is expressed in the cell as discussed herein below. Generally the construct contains

the proper regulatory sequence or promotor to allow the sequence to be expressed in the targeted cell.

The proteins of the present invention can be produced recombinantly (see generally Marshak et al, 1996 "Strategies for Protein Purification and Characterization. A laboratory course manual.", CSHL Press) and analogues can be due to post-translational processing.

The term analogue as used herein is defined as a nucleic acid sequence or protein which has some differences in their amino acid/nucleotide sequences as compared to the native sequence of the sequences disclosed herein. Ordinarily, the analogue is generally at least 70% homologous over any portion that is functionally relevant. In more preferred embodiments the homology is at least 80% and can approach 95% homology to the protein/nucleotide sequence. The amino acid or nucleotide sequence of an analog can differ from that of the primary sequence when at least one residue is deleted, inserted or substituted, but the protein or nucleic acid molecule remains functional. Differences in glycosylation can provide protein analogues.

"Functionally relevant" refers to the biological property of the molecule and in this context means an *in vivo* effector or antigenic function or activity that is directly or indirectly performed by a naturally occurring protein or nucleic acid molecule. Effector functions include, but are not limited to include, receptor binding, any enzymatic activity or enzyme modulatory activity, any carrier binding activity, any hormonal activity, any activity in promoting or inhibiting adhesion of cells to extracellular matrix or cell surface molecules, or any structural role as well as having the nucleic acid sequence encode functional protein and can be expressible. The antigenic functions essentially mean the possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against a naturally occurring protein. Biologically active analogues share an

effector function of the native which can, but do not necessarily, additionally possess an antigenic function.

The above discussion provides a factual basis for the use of the sequences of the present invention to identify bladder cancer-associated genes and provide diagnostic probes and markers to identify bladder cancer, particularly in the early stages of TCC.

EXAMPLES

METHODS

A detailed description of the methods of the present invention are set forth below and are set forth in US patent application USSN 09/534,661 of the applicant company, filed on March 24, 2000 and incorporated herein by reference in its entirety. The method includes preparing cell fractionations; extracting intact total RNA from membrane bound polysomes and free polysomes; preparing cDNA probes from template RNA derived from the extracted polysomes; performing microarray-based comparison of the relative abundance of the different RNA species; analyzing the results; and identifying genes or clones encoding membranal or secreted proteins.

Identification of cDNAs and genes encoding secreted or membranal coding mRNAs is of major importance in TCC. More specifically, novel genes which mark the early stages of TCC and code for secreted proteins are the ultimate markers for diagnosis and follow-up of TCC. By deriving probes from template RNA extracted from membrane-bound polysomes and free polysomes and performing microarray-based comparison of the relative abundance of different RNA species, such potentially secreted proteins can be identified. Analysis of the results of such comparison and identification of the clones encoding for membranal or secreted

proteins provides a valuable tool which can be used together with other gene discovery tools, and which in itself enables identification of likely targets for drug development.

Since membranal and secreted proteins are both accessible and critical for transduction of numerous intra- and intercellular signals, they are generally viewed as preferred targets for pharmacological use and intervention. Therefore, the *a priori* classification of arrayed unknown gene sequences into those that potentially code for secreted and membranal proteins is of great value for the optimization of a high-throughput process of identifying potential drug targets. Furthermore, the identification of genes which express membranal or secreted proteins that are differentially expressed in different cellular situations is of the utmost importance in designing therapeutic or diagnostic tools for TCC.

A method of identifying clones which encode membranal and secreted proteins was employed by preparing bladder cancer cell fractionations, preparing cDNA probes from template RNA derived from membrane-bound polysomes and free-polysomes, performing a microarray-based comparison of the relative abundance of different RNA species, analyzing the results and thereby identifying genes encoding for membranal and secreted proteins. Since membranal and secreted proteins are generally viewed as preferred targets for pharmacological intervention, the present invention thus provides a method of identifying likely targets for TCC diagnosis and therapy.

HYBRIDIZATION AND PROBES:

TCC and normal bladder hybridization:

The probes were prepared from normal healthy bladder samples and from TCC tumors. Only intact RNA with a proper histological report indicating the

existence of TCC was used. All normal and tumor material was collected from two separate clinical centers. Such approach minimizes the influence of local specific surgical bias or subjectivity of the pathological report.

Forty-one hybridizations were performed. In each hybridization, two probes were used simultaneously, each labeled with either Cy3 or Cy5.

These probes were as follows: Probe 1. Probe 1 was common to all hybridizations (common control probe). RNA from TCC samples was mixed with RNA from normal bladder samples. An equal amount of the RNA mixture was labeled with Cy3 and used in all hybridizations; and Probe 2. In each of the hybridizations, a different RNA sample from a single donor was used (test probe).

A common control for all the hybridizations enables comparison of the results between the different hybridizations. If the common control (probe 1) hybridization results are similar in pattern in different hybridizations, comparison can be made between the results of probe 2 hybridizations and all hybridizations.

Seventeen hybridizations included 16 RNA samples extracted from different control healthy bladder mucosa labeled with Cy5. Twenty-three hybridizations were performed with RNA samples derived from tumor tissues, either from non-invasive Ta or from T1 stages of development. Two hybridizations were performed with RNA extracted from 2 invasive TCC samples.

The hybridizations were carried out in three separate sets, but the same common control was used in all sets. Set 1 includes hybridizations 2-11 (TC2A-TC11A), set 2 includes hybridizations 16-25 (TC16A-TC25A), and set 3 includes hybridizations 28-41 (TC28A-TC41A). By using three different sets of hybridizations, the possibility of technical effects related to specific hybridizations is reduced. See Tables below and related description.

Probe from annotation of potentially secreted proteins:

TCC cell line -T24- (from ATCC) was used for cellular fractionation. Membrane-bound polysomes were separated from free polysomes using a sucrose step gradient. RNA coding for potentially secreted proteins was isolated from this microsomal-membranal fraction and separated from RNA coding for intracellular proteins. Hybridization was performed as described hereunder.

The probes used were as follows: Probe 1. Free polysomal RNA fraction labeled with Cy3; and Probe 2. Membrane-bound RNA fraction with Cy5.

TCC CHIP PREPARATION

All hybridizations were performed on TCC designated microarray. The microarray was made up of cDNA clones derived from 3 different libraries:

1. SDGI library: (Described in US Patent Application USSN 09/538,709 of same applicant company, filed 30 March, 2000, and incorporated herein by reference in its entirety): A pool of non-invasive TCC, invasive TCC and normal bladder was used for library preparation. 4550 clones from the SDGI library were included in the TCC chip.
2. Antisense library: (Described in US Provisional Patent Application SN 60/157,843 of same applicant company, filed 6 October, 1999, and incorporated herein by reference in its entirety): The same cDNA pool used for the SDGI library was used for the preparation of a library enriched for antisense sequences. 450 clones from this library were included in the TCC chip.
3. SSH library: (Diatchenko et al., 1996). A subtraction library was made as follows. A normal bladder RNA pool was used for subtraction from non-invasive TCC RNA pool. The subtracted cDNA was used for

the microarray printing. 5000 clones from the SSH library were used for printing.

General methods in molecular biology:

Standard molecular biology techniques known in the art and not specifically described were generally followed as in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York (1989), and in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989) and in Perbal, *A Practical Guide to Molecular Cloning*, John Wiley & Sons, New York (1988), and in Watson et al., *Recombinant DNA*, Scientific American Books, New York and in Birren et al (eds) *Genome Analysis: A Laboratory Manual Series, Vols. 1-4* Cold Spring Harbor Laboratory Press, New York (1998) and methodology as set forth in United States patents 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057 and incorporated herein by reference. Polymerase chain reaction (PCR) was carried out generally as in *PCR Protocols: A Guide To Methods And Applications*, Academic Press, San Diego, CA (1990). In-situ (In-cell) PCR in combination with Flow Cytometry can be used for detection of cells containing specific DNA and mRNA sequences (Testoni et al, 1996, Blood 87:3822.)

General methods in immunology: Standard methods in immunology known in the art and not specifically described are generally followed as in Stites et al.(eds), Basic and Clinical Immunology (8th Edition), Appleton & Lange, Norwalk, CT (1994) and Mishell and Shiigi (eds), Selected Methods in Cellular Immunology, W.H. Freeman and Co., New York (1980).

Immunoassays

In general, ELISAs where appropriate are one of the immunoassays employed to assess a specimen. ELISA assays are well known to those skilled in the art. Both polyclonal and monoclonal antibodies can be used in the assays. Where appropriate other immunoassays, such as radioimmunoassays (RIA) can be used as are known to those in the art. Available immunoassays are extensively described in the patent and scientific literature. See, for example, United States patents 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521 as well as Sambrook et al, *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor, New York, 1989

Antibody Production

Antibodies can be either monoclonal, polyclonal or recombinant. Conveniently, the antibodies can be prepared against the immunogen or portion thereof for example a synthetic peptide based on the sequence, or prepared recombinantly by cloning techniques or the natural gene product and/or portions thereof can be isolated and used as the immunogen. Immunogens can be used to produce antibodies by standard antibody production technology well known to those skilled in the art as described generally in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988 and Borrebaeck, *Antibody Engineering - A Practical Guide*, W.H. Freeman and Co., 1992. Antibody fragments can also be prepared from the antibodies and include Fab, $F(ab')_2$, and Fv by methods known to those skilled in the art.

For producing polyclonal antibodies a host, such as a rabbit or goat, is immunized with the immunogen or immunogen fragment, generally with an adjuvant and, if necessary, coupled to a carrier; antibodies to the immunogen are collected from the sera. Further, the polyclonal antibody can be absorbed such

that it is monospecific. That is, the sera can be absorbed against related immunogens so that no cross-reactive antibodies remain in the sera rendering it monospecific.

For producing monoclonal antibodies the technique involves hyperimmunization of an appropriate donor with the immunogen, generally a mouse, and isolation of splenic antibody producing cells. These cells are fused to a cell having immortality, such as a myeloma cell, to provide a fused cell hybrid which has immortality and secretes the required antibody. The cells are then cultured, in bulk, and the monoclonal antibodies harvested from the culture media for use.

For producing recombinant antibody (see generally Huston et al, 1991; Johnson and Bird, 1991; Mernaugh and Mernaugh, 1995), messenger RNAs from antibody producing B-lymphocytes of animals, or hybridoma are reverse-transcribed to obtain complimentary DNAs (CDNAs). Antibody cDNA, which can be full or partial length, is amplified and cloned into a phage or a plasmid. The cDNA can be a partial length of heavy and light chain cDNA, separated or connected by a linker. The antibody, or antibody fragment, is expressed using a suitable expression system to obtain recombinant antibody. Antibody cDNA can also be obtained by screening pertinent expression libraries.

The antibody can be bound to a solid support substrate or conjugated with a detectable moiety or be both bound and conjugated as is well known in the art. (For a general discussion of conjugation of fluorescent or enzymatic moieties see Johnstone & Thorpe, *Immunochemistry in Practice*, Blackwell Scientific Publications, Oxford, 1982.) The binding of antibodies to a solid support substrate is also well known in the art. (see for a general discussion Harlow & Lane *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Publications, New York, 1988 and Borrebaeck, *Antibody Engineering - A Practical Guide*, W.H. Freeman and Co., 1992) The detectable moieties contemplated with the present

invention can include, but are not limited to, fluorescent, metallic, enzymatic and radioactive markers such as biotin, gold, ferritin, alkaline phosphatase, β-galactosidase, peroxidase, urease, fluorescein, rhodamine, tritium, ^{14}C and iodination.

Recombinant Protein Purification

Marshak et al, "Strategies for Protein Purification and Characterization. A laboratory course manual." CSHL Press, 1996.

Transgenic and Knockout Methods

The present invention provides for transgenic gene and polymorphic gene animal and cellular (cell lines) models as well as for knockout models. These models are constructed using standard methods known in the art and as set forth in United States Patents 5,487,992, 5,464,764, 5,387,742, 5,360,735, 5,347,075, 5,298,422, 5,288,846, 5,221,778, 5,175,385, 5,175,384,5,175,383, 4,736,866 as well as Burke and Olson (1991), Capecchi (1989), Davies et al. (1992), Dickinson et al. (1993), Duff and Lincoln (1995), Huxley et al. (1991), Jakobovits et al. (1993), Lamb et al. (1993), Pearson and Choi (1993), Rothstein (1991), Schedl et al. (1993), Strauss et al. (1993). Further, patent applications WO 94/23049, WO 93/14200, WO 94/06908, WO 94/28123 also provide information.

Further, one parent strain instead of carrying a direct human transgene can have the homologous endogenous gene modified by gene targeting such that it approximates the transgene. That is, the endogenous gene has been "humanized" and/or mutated (Reaume et al, 1996). It should be noted that if the animal and human sequence are essentially homologous a "humanized" gene is not required. The transgenic parent can also carry an overexpressed sequence, either the

nonmutant or a mutant sequence and humanized or not as required. The term transgene is therefore used to refer to all these possibilities.

Additionally, cells can be isolated from the offspring which carry a transgene from each transgenic parent and that are used to establish primary cell cultures or cell lines as is known in the art.

Where appropriate, a parent strain is homozygous for the transgene. Additionally, where appropriate, the endogenous nontransgene in the genome that is homologous to the transgene is nonexpressive. By nonexpressive is meant that the endogenous gene is not expressed and that this nonexpression is heritable in the offspring. For example, the endogenous homologous gene could be "knocked-out" by methods known in the art. Alternatively, the parental strain that receives one of the transgenes could carry a mutation at the endogenous homologous gene rendering it nonexpressed.

For gene therapy:

By gene therapy as used herein refers to the transfer of genetic material (e.g DNA or RNA) of interest into a host to treat or prevent a genetic or acquired disease or condition phenotype. The genetic material of interest encodes a product (e.g. a protein, polypeptide, peptide, functional RNA, antisense) whose production *in vivo* is desired. For example, the genetic material of interest can encode a hormone, receptor, enzyme, polypeptide or peptide of therapeutic value. Alternatively, the genetic material of interest encodes a suicide gene. For a review see, in general, the text "Gene Therapy" (Advances in Pharmacology 40, Academic Press, 1997).

Two basic approaches to gene therapy have evolved: (1) *ex vivo* and (2) *in vivo* gene therapy. In *ex vivo* gene therapy cells are removed from a patient, and while being cultured are treated *in vitro*. Generally, a functional replacement gene

is introduced into the cell via an appropriate gene delivery vehicle/method (transfection, transduction, homologous recombination, etc.) and an expression system as needed and then the modified cells are expanded in culture and returned to the host/patient. These genetically reimplanted cells have been shown to express the transfected genetic material *in situ*.

In *in vivo* gene therapy, target cells are not removed from the subject rather the genetic material to be transferred is introduced into the cells of the recipient organism *in situ*, that is within the recipient. In an alternative embodiment, if the host gene is defective, the gene is repaired *in situ* [Culver, 1998]. These genetically altered cells have been shown to express the transfected genetic material *in situ*.

The gene expression vehicle is capable of delivery/ transfer of heterologous nucleic acid into a host cell. The expression vehicle can include elements to control targeting, expression and transcription of the nucleic acid in a cell selective manner as is known in the art. It should be noted that often the 5'UTR and/or 3'UTR of the gene can be replaced by the 5'UTR and/or 3'UTR of the expression vehicle. Therefore as used herein the expression vehicle can, as needed, not include the 5'UTR and/or 3'UTR of the actual gene to be transferred and only include the specific amino acid coding region.

The expression vehicle can include a promotor for controlling transcription of the heterologous material and can be either a constitutive or inducible promotor to allow selective transcription. Enhancers that can be required to obtain necessary transcription levels can optionally be included. Enhancers are generally any non-translated DNA sequence which works contiguously with the coding sequence (in cis) to change the basal transcription level dictated by the promoter. The expression vehicle can also include a selection gene as described herein below.

Vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989), Chang et al., *Somatic Gene Therapy*, CRC Press, Ann Arbor, MI (1995), Vega et al., *Gene Targeting*, CRC Press, Ann Arbor, MI (1995), *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston MA (1988) and Gilboa et al (1986) and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors. In addition, see United States patent 4,866,042 for vectors involving the central nervous system and also United States patents 5,464,764 and 5,487,992 for positive-negative selection methods.

Introduction of nucleic acids by infection offers several advantages over the other listed methods. Higher efficiency can be obtained due to their infectious nature. Moreover, viruses are very specialized and typically infect and propagate in specific cell types. Thus, their natural specificity can be used to target the vectors to specific cell types *in vivo* or within a tissue or mixed culture of cells. Viral vectors can also be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

A specific example of DNA viral vector for introducing and expressing recombinant sequences is the adenovirus derived vector Adenop53TK. This vector expresses a herpes virus thymidine kinase (TK) gene for either positive or negative selection and an expression cassette for desired recombinant sequences. This vector can be used to infect cells that have an adenovirus receptor which includes most cancers of epithelial origin as well as others. This vector as well as others that exhibit similar desired functions can be used to treat a mixed population of cells and can include, for example, an *in vitro* or *ex vivo* culture of cells, a tissue or a human subject.

Additional features can be added to the vector to ensure its safety and/or enhance its therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells infected with the recombinant virus. An example of such a negative selection marker is the TK gene described above that confers sensitivity to the antibiotic gancyclovir. Negative selection is therefore a means by which infection can be controlled because it provides inducible suicide through the addition of antibiotic. Such protection ensures that if, for example, mutations arise that produce altered forms of the viral vector or recombinant sequence, cellular transformation can not occur.

Features that limit expression to particular cell types can also be included. Such features include, for example, promoter and regulatory elements that are specific for the desired cell type.

In addition, recombinant viral vectors are useful for *in vivo* expression of a desired nucleic acid because they offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. This is in contrast to vertical-type of infection in which the infectious agent spreads only through daughter progeny. Viral vectors can also be produced that are unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

As described above, viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of viral vectors utilizes its natural specificity to specifically target predetermined cell types and thereby introduce a recombinant gene into the infected cell. The vector to be used

in the methods of the invention depends on desired cell type to be targeted and is known to those skilled in the art. For example, if breast cancer is to be treated then a vector specific for such epithelial cells are used. Likewise, if diseases or pathological conditions of the hematopoietic system are to be treated, then a viral vector that is specific for blood cells and their precursors, preferably for the specific type of hematopoietic cell, is used.

Retroviral vectors can be constructed to function either as infectious particles or to undergo only a single initial round of infection. In the former case, the genome of the virus is modified so that it maintains all the necessary genes, regulatory sequences and packaging signals to synthesize new viral proteins and RNA. Once these molecules are synthesized, the host cell packages the RNA into new viral particles which are capable of undergoing further rounds of infection. The vector's genome is also engineered to encode and express the desired recombinant gene. In the case of non-infectious viral vectors, the vector genome is usually mutated to destroy the viral packaging signal that is required to encapsulate the RNA into viral particles. Without such a signal, any particles that are formed do not contain a genome and therefore cannot proceed through subsequent rounds of infection. The specific type of vector depends upon the intended application. The actual vectors are also known and readily available within the art or can be constructed by one skilled in the art using well-known methodology.

The recombinant vector can be administered in several ways. If viral vectors are used, for example, the procedure can take advantage of their target specificity and consequently, do not have to be administered locally at the diseased site. However, local administration can provide a quicker and more effective treatment, administration can also be performed by, for example, intravenous or subcutaneous injection into the subject. Injection of the viral vectors into a spinal fluid can also be used as a mode of administration, especially in the case of neuro-degenerative diseases. Following injection, the viral vectors circulate until they recognize host cells with the appropriate target specificity for infection.

An alternate mode of administration can be by direct inoculation locally at the site of the disease or pathological condition or by inoculation into the vascular system supplying the site with nutrients or into the spinal fluid. Local administration is advantageous because there is no dilution effect and, therefore, a smaller dose is required to achieve expression in a majority of the targeted cells. Additionally, local inoculation can alleviate the targeting requirement required with other forms of administration since a vector can be used that infects all cells in the inoculated area. If expression is desired in only a specific subset of cells within the inoculated area, then promoter and regulatory elements that are specific for the desired subset can be used to accomplish this goal. Such non-targeting vectors can be, for example, viral vectors, viral genome, plasmids, phagemids and the like. Transfection vehicles such as liposomes can also be used to introduce the non-viral vectors described above into recipient cells within the inoculated area. Such transfection vehicles are known by one skilled within the art.

Delivery of gene products/therapeutics (compound):

The compound of the present invention is administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, patient age, sex, body weight and other factors known to medical practitioners. The pharmaceutically "effective amount" for purposes herein is thus determined by such considerations as are known in the art. The amount must be effective to achieve improvement including but not limited to improved survival rate or more rapid recovery, or improvement or elimination of symptoms and other indicators as are selected as appropriate measures by those skilled in the art.

In the method of the present invention, the compound of the present invention can be administered in various ways. It should be noted that it can be

administered as the compound or as pharmaceutically acceptable salt and can be administered alone or as an active ingredient in combination with pharmaceutically acceptable carriers, diluents, adjuvants and vehicles. The compounds can be administered orally, subcutaneously or parenterally including intravenous, intraarterial, intramuscular, intraperitoneally, and intranasal administration as well as intrathecal and infusion techniques. Implants of the compounds are also useful. The patient being treated is a warm-blooded animal and, in particular, mammals including man. The pharmaceutically acceptable carriers, diluents, adjuvants and vehicles as well as implant carriers generally refer to inert, non-toxic solid or liquid fillers, diluents or encapsulating material not reacting with the active ingredients of the invention.

It is noted that humans are treated generally longer than the mice or other experimental animals exemplified herein which treatment has a length proportional to the length of the disease process and drug effectiveness. The doses can be single doses or multiple doses over a period of several days, but single doses are preferred.

The doses can be single doses or multiple doses over a period of several days. The treatment generally has a length proportional to the length of the disease process and drug effectiveness and the patient species being treated.

When administering the compound of the present invention parenterally, it is generally formulated in a unit dosage injectable form (solution, suspension, emulsion). The pharmaceutical formulations suitable for injection include sterile aqueous solutions or dispersions and sterile powders for reconstitution into sterile injectable solutions or dispersions. The carrier can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils.

Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Nonaqueous vehicles such a cottonseed oil, sesame oil, olive oil, soybean oil, corn oil, sunflower oil, or peanut oil and esters, such as isopropyl myristate, can also be used as solvent systems for compound compositions. Additionally, various additives which enhance the stability, sterility, and isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. In many cases, it is desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the present invention, however, any vehicle, diluent, or additive used have to be compatible with the compounds.

Sterile injectable solutions can be prepared by incorporating the compounds utilized in practicing the present invention in the required amount of the appropriate solvent with various of the other ingredients, as desired.

A pharmacological formulation of the present invention can be administered to the patient in an injectable formulation containing any compatible carrier, such as various vehicle, adjuvants, additives, and diluents; or the compounds utilized in the present invention can be administered parenterally to the patient in the form of slow-release subcutaneous implants or targeted delivery systems such as monoclonal antibodies, vectored delivery, iontophoretic, polymer matrices, liposomes, and microspheres. Examples of delivery systems useful in the present invention include: 5,225,182; 5,169,383; 5,167,616; 4,959,217; 4,925,678; 4,487,603; 4,486,194; 4,447,233; 4,447,224; 4,439,196; and 4,475,196. Many other such implants, delivery systems, and modules are well known to those skilled in the art.

A pharmacological formulation of the compound utilized in the present invention can be administered orally to the patient. Conventional methods such as administering the compounds in tablets, suspensions, solutions, emulsions, capsules, powders, syrups and the like are usable. Known techniques which deliver it orally or intravenously and retain the biological activity are preferred. In one embodiment, the compound of the present invention can be administered initially by intravenous injection to bring blood levels to a suitable level. The patient's levels are then maintained by an oral dosage form, although other forms of administration, dependent upon the patient's condition and as indicated above, can be used. The quantity to be administered vary for the patient being treated and vary from about 100 ng/kg of body weight to 100 mg/kg of body weight per day and preferably are from 10 μg/kg to 10 mg/kg per day.

Differential Analysis

Appropriate cells, cell lines or tissues are grown under normal conditions (non-stress) or under stress conditions generally for four to sixteen hours. Alternatively two cell types are compared. The cells are harvested and RNA is prepared from the cytoplasmic extracts and from the nuclear fractions. Following the extraction of RNA, fluorescent cDNA probes are prepared. Each condition is labeled with a different fluorescent dye. For example a probe can be composed of a mixture of Cy3 -dCTP cDNA prepared from RNA extracted from stressed cells and with Cy5-dCTP cDNA prepared from RNA extracted from nonstressed cells. The probes are used for hybridization to micro-array containing individually spotted cDNA clones derived from cells that were exposed to stress. Differential expression in measured by the amount of fluorescent cDNA that hybridizes to each of the clones on the array. Genes that are up regulated under stress have more fluorescence of Cy3 than Cy5. The results generally show genes that are transcriptionally induced mRNA species that respond very fast to stress.

Differential display:

Reverse transcription: 2μg of RNA are annealed with 1pmol of oligo dT primer $(dT)_{18}$ in a volume of 6.5μl by heating to 70° C for five minutes and cooling on ice. 2μl reaction buffer (x5), 1μl of 10mM dNTP mix, and 0.5μ1 of SuperScript II reverse transcriptase (GibcoBRL) is added. The reaction is carried for one hour at 42°C. The reaction is stopped by adding 70μl TE (10mM Tris pH=8; 0.1mM EDTA).

Oligonucleotides were used for Differential display. The oligonucleotides are generally those described in the Delta RNA Fingerprinting kit (Clonetech Labs. Inc.).

Amplification reactions: Each reaction is done in 20μl and contains 50μM dNTP mix, 1μM from each primer, 1x polymerase buffer, 1 unit expand Polymerase (Beohringer Mannheim), 2μCi [α-^{32}P]dATP and 1μl cDNA template. Cycling conditions are generally: three minutes at 95°C, then three cycles of two minutes at 94°C, five minutes at 40°C, five minutes at 68°C. This is followed by 27 cycles of one minute at 94°C, two minutes at 60°C, two minutes at 68°C. Reactions were terminated by a seven minute incubation at 68°C and addition of 20μl sequencing stop solution (95% formamide, 10mM NaOH, 0.025% bromophenol blue, 0.025% xylene cyanol).

Gel analysis: Generally 3-4μl are loaded onto a 5% sequencing polyacrylamide gel and samples are electrophoresed at 2000 volts/40 milliamperes until the slow dye (xylene cyanol) is about 2 cm from the bottom. The gel is transferred to a filter paper, dryed under vacuum and exposed to x-ray film.

Recovery of differential bands: Bands showing any a differential between the various pools are excised out of the dried gel and placed in a microcentrifuge

tube. 50µl of sterile H_2O are added and the tubes heated to 100°C for five minutes. 1µl is added to a 49µl PCR reaction using the same primers used for the differential display and the samples are amplified for 30 cycles of: one minute at 94°C, one minute at 60°C and one minute at 68°C. 10µl is analyzed on agarose gel to visualize and confirm successful amplification.

Representational difference analysis

Reverse transcription: as above but with 2µg polyA+ selected mRNA.

Preparation of double stranded cDNA: cDNA from the previous step is treated with alkali to remove the mRNA, precipitated and dissolved in 20µl H_2O. 5µl buffer, 2µl 10mM dATP, H_2O to 48µl and 2µl terminal deoxynucleotide transferase (TdT) are added. The reaction is incubated two to four hours at 37°C. 5µl oligo dT (1µg/µl) was added and incubated at 60°C for five minutes. 5µl 200 mM DTT, 10 µl 10x section buffer (100mM Mg $C1_2$, 900 mM Hepes, pH 6.6) 16 µl dNTPs (1 mM), and 16 U of Klenow are added and the mixture incubated overnight at room temperature to generate ds cDNA. 100µl TE is added and extracted with phenol/chloroform. The DNA is precipitated and dissolved in 50µl H_2O.

Generation of representations: cDNA with DpnII is digested by adding 3µl DpnII reaction buffer 20 V and DpnII to 25µl cDNA and incubated five hours at 37° C. 50µl TE is added and extracted with phenol/chloroform. cDNA is precipitated and dissolved to a concentration of 10ng/µl.

Driver: 1.2µg DpnII digested cDNA. 4µl from each oligo and 5µl ligation buffer x10 and annealed at 60°C for ten minutes. 2µl ligase is added and incubated overnight at 16°C. The ligation mixture is diluted by adding 140µl TE. Amplification is carried out in a volume of 200µl using appropriate primer and 2µl ligation product and repeated in twenty tubes for each sample. Before adding Taq

DNA polymerase, the tubes are heated to 72°C for three minutes. PCR conditions are as follows: five minutes at 72°C, twenty cycles of one minute at 95°C and three minutes at 72°C, followed by ten minutes at 72°C.

Every four reactions were combined, extracted with phenol/chloroform and precipitated. Amplified DNA is dissolved to a concentration of 0.5μg/μl and all samples are pooled.

Subtraction: Tester DNA (20μg) is digested with DpnII as above and separated on a 1.2% agarous gel. The DNA is extracted from the gel and 2μg ligated to the appropriate oligos. The ligated Tester DNA is then diluted to 10ng/μl with TE. Driver DNA is digested with DpnII and repurified to a final concentration of 0.5μg/μl. Mix 40μg of Driver DNA with 0.4μg of Tester DNA. Extraction is carried out with phenol/chloroform and precipitated using two washes with 70% ethanol, resuspended DNA in 4μl of 30mM EPPS pH=8.0, 3mM EDTA and overlaid with 35μl mineral oil. Denature at 98°C for five minutes, cool to 67°C and 1μl of 5M NaCl added to the DNA. Incubate at 67°C for twenty hours. Dilute DNA by adding 400μl TE.

Amplification: Amplification of subtracted DNA in a final volume of 200μl as follows: Buffer, nucleotides and 20μl of the diluted DNA are added, heated to 72° C, and Taq DNA polymerase added. Incubate at 72°C for five minutes and add apprpriate oligo. Ten cycles of one minute at 95°C, three minutes at 70°C are performed. Incubate ten minutes at 72°C. The amplification is repeated in four separate tubes. The amplified DNA is extracted with phenol/chloroform, precipitated and all four tubes combined in 40μl 0.2xTE, and digested with Mung Bean Nuclease as follows: To 20μ1 DNA 4μl buffer, 14μl H_2O and 2μl Mung Bean Nuclease (10 units/μl) added. Incubate at 30°C for thirty-five minutes + First Differential Product (DPI).

Repeat subtraction hybridization and PCR amplification at driver: differential ratio of 1:400 (DPII) and 1:40,000 (DPIII) using appropriate oligonucleotides. Differential products are then cloned into a Bluescript vector at the BAM HI site for analysis of the individual clones.

DIFFERENTIAL EXPRESSION USING GENE EXPRESSION MICRO-ARRAY

Messenger RNA isolated as described herein above is labeled with fluorescent dNTP's using a reverse transcription reaction to generate a labeled cDNA probe. mRNA is extracted from cells cultured in non-stress conditions and labeled with Cy3-dCTP (Amersham) and mRNA extracted from cells cultured under stress conditions is labeled with Cy5-dCTP (Amersham). The two labeled cDNA probes are then mixed and hybridized onto a microarray (Schena et al, 1996) composed of for example 2000 cDNA clones derived from a cDNA library prepared from appropriate cells cultured under the stress conditions. Following hybridization the microarray is scanned using a laser scanner and amount of fluorescence of each of the fluorescence dyes is measured for each cDNA clone on the micro-array giving an indication of the level of mRNA in each of the original mRNA populations being tested. Comparison of the fluorescence on each cDNA clone on the micro-array between the two different fluorescent dyes is a measure for the differential expression of the indicated genes between the two experimental conditions.

IN SITU ANALYSIS

In situ analysis as needed is performed for the candidate genes identified by the methods as described above.

Cell culture protocols.

C6, Hela and Jurkat cell lines (ATCC) were grown either in DMEM (C6 and HeLa) or in RPMI (Jurkat) supplemented with 10% FCS. C6 cells were grown either under normal oxygen conditions or under hypoxia for 4 or 16 hours (0.5%O_2 and 5%CO_2). Jurkat cells were grown either at 37°C or exposed to 43°C for one or four hours. HeLa cells were kept under normal tissue culture conditions. One to three hours prior to harvesting (for non-treated cells) or prior to treatment, culture medium was replaced with a fresh one. Cells were then washed with ice cold PBS-/-, harvested on ice and either directly processed to extraction of total RNA or collected by centrifugation (400g, 5 minutes). The cell pellet was immediately frozen in liquid nitrogen and kept at -70° until used for fractionation to isolate nuclei, polysomes or microsomes.

Cell fractionation protocols.

Preparation of nuclear and polysomal fractions. Nuclear and polysomal subcellular fractions were obtained in a single fractionation procedure as previously described [23]. Briefly, frozen cell pellets were resuspended in ice-cold polysomal buffer: 25mM Tris HCl, pH 7.4-7.5; 10mM $MgCl_2$; 25mM NaCl; 0.05% Triton X-100; 0.14M sucrose, 100 μg/ml heparin, 1mM DTT and 133 μg/ml RNasin (Promega). 0.1% Triton X-100 and 0.1% deoxycholate were added and following 3' incubation on ice, the nuclei were pelleted (10,000g, 4°C, 3') and NUC RNA was extracted . The supernatant was further processed for isolation of the polysomal fraction. After addition of 1mg/ml of heparin it was layered on top of a sucrose gradient (0.5M - 1.5M) and centrifuged (Beckman, SW41, 36K, 4°C, 110'). Upon centrifugation, fractions were collected into tubes containing 0.5% SDS. The polysomal fraction was further used for extraction of RNA

Preparation of membrane bound and free polysomes. For isolation of microsomes, Hela cells were grown to 50%-70% confluence and Jurkat cells were grown to cell densities 0.5 - 1X10^6 cells/ml. Cytoplasmic lysate was prepared essentially as

described[24]. Briefly, Hela or Jurkat cell pellets were thawed on ice and lysed with 0.25M sucrose lysis buffer (250mM sucrose, 50mM TEA (triethanolamine), 50mM KOAc pH 7.5, 6mM Mg(OAc)2, 1mM EDTA, 1mM DTT, 0.5mM PMSF, 200 units/ml RNasine (Promega), 0.1mg/ml heparin) - 1ml per 10^8 cells. Cells were homogenized on ice using a Teflon homogenizer (Heidolph, 1000 rpm). Following centrifugation (600g, 10 minutes, 4°C), the supernatant was collected and kept on ice. The nuclei pellet was washed with 1ml of lysis buffer and re-centrifuged (600g, 10 minutes, 4°C) to release the contaminating microsomes. The resultant supernatant was combined with the previous one. Total supernatant material was further centrifuged to precipitate mitochondria (10,000g, 10 minutes, 4°C) and heparin (1mg/ml) was added to the collected supernatant. The latter (5 ml) was layered over the two-step sucrose gradient (2.5 ml of 2.05M sucrose in lysis buffer/2.5 ml of 1.5M sucrose in lysis buffer). After centrifugation (100,000g, 3 hours, 4°C), the upper phase (5 ml) containing mRNPs was discarded. Middle phase (2.5 ml) containing membrane bound polysomes (MBP) and pellet containing free polysomes (FP) were further used for RNA extraction.

RNA extraction protocols

Extraction of total cellular (TC) and nuclear RNA (NUC) was performed with Tri-Reagent (Sigma) according to commercial protocols.

Extraction of RNA from membrane bound (MBP) and free polysomes (FP). Pellet of free polysomes was dissolved in 1% SDS/0.1M NaCl. Membrane polysome fraction was diluted with TE 1:1 and SDS and proteinase K (Sigma) were added to final concentrations 1% and 0.1mg/ml, respectively. Following incubation at 37°C for 30 minutes, RNA was purified from both fractions with phenol:chloroform (1:1) and precipitated with 0.3M NaOAc, 20μg/ml glycogen (Roche) and 50% isopropanol. Following centrifugation (10,000g, 20 minutes, 4°C), RNA pellets were washed with 70% ethanol, dried and dissolved in H_2O.

Extraction of polysomal RNA (POL) was done as described for the MBP fraction.

cDNA microarrays.

C6-specific microarray. A cDNA microarray containing 1847 cDNA fragments was constructed from clones of subtracted cDNA libraries derived from C6 glioma cells enriched for hypoxia-responsive mRNAs. Specifically, mRNA prepared from C6 cells cultured under hypoxic conditions for 4 and 16 hours, respectively, was subjected to bidirectional subtraction (PCR-Select cDNA subtraction kit, Clontech) followed by cloning into pBluescript. Three sublibraries were prepared. Sublibrary A was enriched for cDNA species up-regulated after four hours of hypoxia: sublibrary B, for cDNA species up-regulated after 16 hours of hypoxia; and sublibrary C, for cDNA species down-regulated after 16 hours of hypoxia. For microarray printing, 523 cDNA clones were selected from sublibrary A, 884 from sublibrary B, and 373 from sublibrary C. The array also contained a set of control genes whose response to hypoxia in C6 glioma (and other cells) is well documented including vascular-endothelial growth factor (VEGF)[8,9,25], glucose transporter 1(Glut1)[9,26] and lactate dehydrogenase (LDH)[27].

F2054-specific microarray. This microarray contained 1418 human cDNA clones collected from a library derived from the human F2054 fibroblast cell line. The library was prepared in pBluescript, the average insert size being about 500 bp.

Human UniGEM1 microarray (Incyte). This commercially available microarray that contains 9,700 human cDNA clones, 60% of them coding for known genes, was used in experiments with MBP and FP derived probes. Since proper interpretation of experimental data requires precise clone identity, only clones that were verified by sequencing in Incyte (2,800 out of 9,700) were subjected to the analysis.

Probe labeling and hybridization to cDNA microarrays

cDNA probes were synthesized from 50μg of RNA using reverse transcriptase (Superscript, Gibco-BRL) and 18-mer oligo-dT primer. The hybridization probe was composed of two cDNA populations, derived from two different RNA sources: one labelled with Cy3-dCTP and the other with Cy5-dCTP (Amersham), as previously described[28]. The following types of probes were used for hybridizations to different DNA microarrays:

C6-specific microarray. This microarray was utilized for hybridizations of C6-derived probes: (1) total RNA normoxia (Cy3)/total RNA four hours hypoxia (Cy 5); (2) total RNA normoxia (Cy 3)/ total RNA 16 hours hypoxia (Cy 5); (3) nuclear RNA normoxia (Cy3)/nuclear RNA 4 hours hypoxia (Cy5); (4) nuclear RNA normoxia (Cy3)/nuclear RNA 16 hrs hypoxia (Cy5).

F2054-specific microarray. This microarray was utilized for hybridization of probes derived from heat shock experiments with Jurkat cells: (1)total RNA 37°C(Cy3)/total RNA 1 hour 43°C(Cy 5); (2) total RNA 37°C (Cy 3)/ total RNA four hours 43°C (Cy 5); (3) nuclear RNA 37°C (Cy3)/nuclear RNA 1hr 43°C (Cy5); (4) polysomal RNA 37°C (Cy3)/polysomal RNA 1 hour 43°C (Cy5); (5) polysomal RNA 37°C (Cy3)/polysomal RNA four hours 43°C (Cy5)

Unigem1(Incyte). This microarray was utilized for hybridization of probes derived from different polysomal fractions: (1) MBP RNA from Hela (Cy3)/ FP RNA from Hela (Cy5); (2) MBP RNA from Jurkat (Cy3)/ FP RNA from Jurkat (Cy5).

Hybridization data processing

Hybridizations, image processing and signal calculation were performed using commercial tools (Incyte, GEMTools). Overall signals were balanced by this software and differential expression values were calculated as "Cy3 signal" (P1)/"balanced Cy5 signal" (P2) (P1>P2) and "balanced Cy5 signal" (P2)/"Cy3

signal" (P1) (P2>P1). For this reason, the numerical values shown in the tables may vary from the observed images. For all differentially expressed clones the image data was visually inspected. Since there was observed a certain inconsistency of hybridization results obtained with low expressed cDNA clones, in all experiments only clones that displayed an hybridization signal not lower than 500 units, at least in one of the fluorescence channels, were subjected to further analysis.

Analysis of discrimination of MBP and FP cDNA populations

Out of the annotated expressed clones, a random sample of 118 clones was selected and the subcellular localization of their encoded proteins was assigned. According to the literature and the SWISS-PROT database[29], 29 of them code for either membranal/secreted or ribosomal proteins (MBP-resident), 63 code for cytosolic or nuclear proteins (FP-resident), and the remaining 26 genes could not be clearly assigned. Frequency histograms were built for ln(P1signal/P2 Balanced Signal) since these values are anticipated to be approximated well by a normal distribution (while differential expression values are not). The parameters of normal distributions were then estimated for each histogram according to standard procedures, using the SPSS package, which was also used for the standard statistical tests for inequality of means of the approximated normal distributions (t-test).

Determination of conditional probabilities for cDNA clones to correspond to the membranal/secreted or to the cytosolic/nuclear group of proteins

In order to calculate the conditional probabilities, we first estimated the *a priori* probability was first estimated for a selected clone to be MBP-resident ($\alpha 1$ in equations (1,2)). This estimation is required since the relative portions of the two clone populations both on the array and in the expressed RNA population are probably not equal (more cytosolic/nuclear clones are expected), thereby biasing in advance their probability of detection. This *a priori* probability for a protein to be MBP-resident ($\alpha 1=0.34$, equation (1)) was calculated based on the assignment of

proteins with prominent differential values >0.69 (right tail, corresponding to >+2 differential expression values) and <-0.69 (left tail, corresponding to <-2 differential expression values) of the distribution, as given in Table 2, so that a combined distribution would have these pre-determined right and left tail frequencies. The parameter $\alpha 1$ was found as a solution of the equation:

(1)

$$FL1 + FR1 = \frac{\alpha 1 ?(1 - \Phi(\frac{w - m1}{SD1}))}{\alpha 1 ?(1 - \Phi(\frac{w - m1}{SD1})) + (1 - \alpha 1) ?(1 - \Phi(\frac{w - m2}{SD2}))} + \frac{\alpha 1 ?\Phi(\frac{-w - m1}{SD1})}{\alpha 1 ?\Phi(\frac{-w - m1}{SD1}) + (1 - \alpha 1) ?\Phi(\frac{-w - m2}{SD2})}$$

where w = 0.69, m1,m2 - means of the two distributions, SD1,SD2 - their standard deviations, Φ is standard normal distribution function (i.e. $\Phi(y) = \frac{1}{\sqrt{2\pi}} \int_{-x}^{y} \exp(-\frac{x^2}{2}) dx$), FL1 (FR1) - the frequency of first kind (MBP- resident) proteins to have prominent differential values less than –w (more than w), FL2 (FR2) - the frequency of second kind (FP-resident) proteins to have prominent differential values less than –w (more than w).

The conditional probabilities for proteins of the first kind (encoded by the MBP-resident mRNAs) and second kind (encoded by the FP-resident mRNAs) (p1 and p2, respectively) under assumption on normality of distributions were calculated according to the following equation:

(2)

$$p1(w) = \frac{\alpha 1 ?\exp(-\frac{(w - m1)^2}{2(SD1)^2})}{\alpha 1 ?\exp(-\frac{(w - m1)^2}{2(SD1)^2}) + (1 - \alpha 1) ?\exp(-\frac{(w - m2)^2}{2(SD2)^2})}$$

where w = ln(P1signal/P2BalancedSignal) and $\alpha 1$ is the *a priori* probability for a protein to be of the first kind.

Bioinformatics sequence analysis of selected EST clones

The EST sequences were extended using QBI's propriety clustering software (manuscript in preparation). Putative coding regions for the obtained contig sequences were defined by two criteria: (1) open reading frame (ORF) longer than 50 amino acids, flanked by untranslated region(s) containing multiple stop codons in all three frames; and (2) when a potential 5' UTR was observed, the presence of an initiation methionine was required; (it was sometimes possible to define it relative to the poly-A tail). The contigs were characterized in two steps. First, homology searches (BLAST) were performed to identify homologous sequences in the nucleotide and protein non-redundant (nr) databases of Genbank. Then the contig sequences were characterized by prediction of motifs and domains using SMART[30], prediction of intracellular localization using PSORT[31], searching for homologous domains in the ProDom database[32], and searching for homologous motifs in ProSite database[33].

RESULTS

Utilizing the methods set forth herein above, the sequences set forth herein were identified and cloned as being differentially expressed in bladder cancer. In both tables, hybridizations are compared.

The sequences named in Table I are identified by clone number and accession number. This list includes sequences of known genes whose function in bladder cancer was heretofore unknown and which were now found to upregulated in bladder cancer. Nucleic acid sequences are provided in Table III.

The sequences named in Table II are identified by clone number. This list includes sequences of novel genes which have no identity to known proteins or genes in the gene databases. Nucleic acid sequences are provided in Table IV.

In both Tables I and II, the differential expression pattern of the different hybridization probes is provided. In both Table I and II, the genes listed were found to be upregulated in at least 60% of TCC samples and unchanged in at least 75% of the normal samples.

Table I and II show the genes as described in biological NCBI databases, with the Genebank number of each gene as presented in the NCBI database. The location of the clone in the TCC microarray of the present invention is set forth in the tables, with their clone ID and the location of the clone in the TCC microarray.

The expression differentials described in Tables I and II were calculated as follows: Since a common control probe was used for all hybridizations and the hybridizations were carried out in three separate sets, the expression differentials in each respective set were calculated as compared to one of the normal bladder samples, as a reference probe.

Thus, hybridization set 1 (Columns 5-14) which includes hybridizations TC2A-TC11A, show all the results as compared to the TC7A (normal) hybridization result. The results are the calculated differential expression (see above for explanation of the calculations) of normal urothel samples (samples designated TC7A-TC11A) and of TCC samples (samples designated TC2A-TC6A).. In hybridization set 2 which includes hybridizations TC16A-TC25A, all the results were calculated in comparison to the TC22A (normal) hybridization result. Set 2 is shown in columns 15-24 wherein there is shown the calculated differential expression (see above for explanation of the calculations) of normal urothel samples (samples designated TC21A-TC24A) and of TCC samples (samples designated TC16A-TC20A and TC25A). In set 3, which includes hybridizations TC28A-TC41A, all the results were calculated compared to the reference normal probe from TC47A. In set 3, as shown in Columns 25-45, the results are the calculated differential expression (see above for explanation of the calculations) of normal urothel samples (samples designated TC35A-TC38A and samples TC46A-

TC48A) and of TCC samples (samples designated TC28A-TC34A and TC39-TC45A).

The last column of Tables I and II show the differential expression of secreted membranal protein identification probe. The smaller the number in the result (negative number), the higher probability for such a gene to code for a secreted or membranal protein.

The first set of raw data show the description of the grade of the TCC tumor i.e. G1-G3 or high / low grade (when available). The second set of raw data are for tumor material, this shows the description of the stage of the tumor i.e. Ta, T1, TIS (tumor in situ) or INV (invasive TCC). For normal urothel samples there is indicated the type of sample (e.g. normal).

All the genes listed in these tables (1 and 2) can be classified as potential markers for non invasive transitional cell carcinoma. As for invasive TCC some of the genes also have diagnostic capabitily for advanced forms of invasive TCC, etc. This approach further enables the identification of potentially secreted molecules which can be used for detection of secreted proteins in urine of TCC patients. Each of the genes listed as potential marker for TCC can also be used for diagnosis of TCC in exfoliated cells in the urine by utilizing RT-PCR technology for the estimation of the level of expression of each of these genes in these cells.All the listed genes in table 1 and 2 can either be used individually or in combination (2-3 genes simultaneously) for more efficient diagnosis of all possible cases of TCC.

Throughout this application, various publications, including United States patents, are referenced by author and year and patents by number. Full citations for the publications are listed below. The disclosures of these publications and patents in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the described invention, the invention can be practiced otherwise than as specifically described.

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TABLE 1

	GeneDescription and clone ID	Accession	GeneID	G2/3 T1 TC2A	G2/3 T1 TC3A	G2/3 T1 TC4A	G2/3 T1 TC5A	G1/2 T1 TC6A	norma TC7A	normal TC8A	normal TC9A	normal TC10A	normal TC11A	G2 T1 TC16A	G2 Ta TC17A	High Tx TC18A	Low T1 TC19A	G3 T1 TC20A
1	Homo sapiens keratin 19 (KRT19) gene, complete cd; nt_non_genomic(identity):47_TCC_91B11_M13F.TXT.fa	g|6729880|gb|AF202321.1|AF202321	TCC-91B_11	3.7	3	5.4	1.5	3.1	1	-2.5	-2	1.2	-1.2	2.6	2.4	2.5	2.6	1.7
2	Human 40-kDa keratin intermediate filament precur; nt_non_genomic(identity):20_TCC_60H4_M13F.TXT.fa	g|184658|gb|J03607.1|HUMIFP	TCC-60H_4	3.7	2.7	5.9	1.5	2.9	1	-2.6	-1.9	1.3	-1.1	2.4	2.6	3.6	2.9	1.6
3	Human mRNA fragment for mesothelial type II keral; nt_non_genomic(identity):04_TCC_94G3_M13F.TXT.fa	g|340067|emb|X03212.1|H8KER7R	TCC-94G_3	3.3	2.7	5.3	3.1	1.6	1	-2.1	-1.6	1.3	-1.4	7.1	4.6	6.3	4.1	4.3
4	Homo sapiens cystatin B (CSTB) gene, promotor reg; nt_non_genomic(identity):30_TCC_76B3_M13F_F04_042.ab1.TXT	g|7263011|gb|AF208234.1|AF208234	TCC-76B_3	3.7	2.2	3	1.6	1.3	1	-2	-1.6	-1	-1.2	3.6	11	5.9	2.3	3.4
5	Homo sapiens S100 calcium-binding protein P (S100P; nt_non_genomic(identity):29_TCC_48G1_M13F.TXT.fa	g|5174682|ref|NM_005980.1|	TCC-48G_1	2.7	4.6	6.9	1.7	1.7	1	-2.6	-2.5	2.1	1.6	3	6.1	6.2	2.6	1.3
6	Homo sapiens syndecan 1 (SDC1) mRNA; nt_non_genomic(identity):26_TCC_75E3_M13F_B04_032.ab1.TXT	g|4508958|ref|NM_002997.1|	TCC-75E_3	1.9	2.6	2.5	1	1.6	1	-1.7	-1.3	-1.1	-1.6	5	4.6	4	2.4	1.7
7	Homo sapiens S100 calcium-binding protein A13 (S10; nt_non_genomic(identity):28_TCC_44C1_M13F.TXT.fa	g|5174683|ref|NM_005979.1|	TCC-44C_1	3.1	4.6	9.3	1.6	1.6	1	-2.6	-2.4	1.9	1.4	2.6	4.9	6.6	2.5	1.1
8	Homo sapiens mRNA for hepatocyte growth factor ac; nt_non_genomic(identity):35_TCC_21D6_M13F_C05_037.ab1.fa	g|2924619|dbj|AB006534.1|AB006534	TCC-21D_6	1.1	1.1	-2.2	2	1.6	1	-1.6	-1.2	1.1	-1.5	2.7	2.6	2.6	1.7	2
9	Homo sapiens midkine (neurite growth-promoting fac; nt_non_genomic(identity):62_TCC_66G3_M13F_B11_092.ab1.TXT	g|4505134|ref|NM_002391.1|	TCC-66G_3	2.2	1.7	3.7	-1.5	1.9	1	-1.2	1.2	-1.6	-1.2	1.9	2.5	1.9	1.4	2.9
10	Homo sapiens solute carrier family 2 (facilitated; nt_non_genomic(identity):07_TCC_57B3_M13F.TXT	g|5730050|ref|NM_006516.1|	TCC-57B_3	4.6	1.2	2.2	1.2	3.2	1	-2.1	-1.6	1.1	-1.6	2.7	1.6	2.9	-1	1.4
11	Homo sapiens S100 calcium-binding protein A11 (cal; nt_non_genomic(identity):31_TCC_65B9_M13F.TXT.fa	g|5032058|ref|NM_005620.1|	TCC-65B_9	3.5	2	3.4	1.5	1.6	1	-1.4	-1.7	1.1	-1.1	2.9	3.6	2.7	2.3	1.6
12	Homo sapiens fatty acid binding protein 5 (psorias; nt_non_genomic(identity):11_TCC_25F2_M13F.TXT	g|4557680|ref|NM_001444.1|	TCC-25F_2	2.1	2.3	1.1	2.6	6	1	-1.6	-1.5	2	1	1.2	1.7	4.6	2.2	1.6
13	Homo sapiens Ops-interacting protein OIP3 mRNA, p; nt_non_genomic(identity):38_TCC_56E11_M13F.TXT	g|2815606|gb|AF025439.1|AF025439	TCC-56E_11	3.4	2.1	2.4	1.1	1.6	1	-1.1	-1.1	-1.3	1.1	2.6	3.6	4.5	2.3	1.7
14	Homo sapiens glutaminyl-tRNA synthetase (QARS), m; nt_non_genomic(identity):46_TCC_78B11_M13F_F08_058.ab1.TXT	g|4826958|ref|NM_005051.1|	TCC-78B_11	2.2	2.6	5.3	1.6	1.6	1	-1.1	-1.3	1.1	1.1	2.7	5	2.1	1.9	1.0
15	Homo sapiens anterior gradient 2 (Xenopus laevis); nt_non_genomic(identity):25_TCC_50G5_M13F.TXT	g|5453640|ref|NM_006408.1|	TCC-50G_5	2.4	5.6	3.4	-1.2	1.2	1	-2	-1.9	1.5	1.2	2.6	2.6	2.9	4.9	1.6
16	Homo sapiens myristoylated alanine-rich protein k; nt_non_genomic(identity):10_TCC_53H11_T3	g|4505082|ref|NM_002356.1|	TCC-53H_11	1.6	1	-1.6	1.6	1.7	1	-1.3	1.3	1.6	1.1	1.9	1.6	2.5	1.5	2.2
17	Homo sapiens leukemia-associated phosphoprotein p1; nt_non_genomic(identity):46_TCC_27H5_M13F_F08_056.ab1.fa	g|5031850|ref|NM_005563.1|	TCC-27H_5	1.6	1.7	2	2.2	1.5	1	-1.2	1.2	-1.1	1.1	-1.1	-1.4	1.4	-1.4	1.2
18	Homo sapiens type II membrane serine protease (LD; nt_non_genomic(identity):53_TCC_79G2_M13_FE07_054.ab1.TXT	g|7705976|ref|NM_016425.1|	TCC-79G_2	5.6	2.6	7.2	-1.6	-1.2	1	-2.2	-2.1	1.1	-1.2	3.6	1.4	3.6	3.9	-1.2
19	Homo sapiens putative secreted protein XAG mRNA, c; nt_non_genomic(identity):26_TCC_60G6_M13F.TXT	g|5852811|gb|AF088867.1|AF088867	TCC-60G_6	2.3	5.7	3.6	-1.1	1.3	1	-1.6	-1.9	1.4	1.2	3.1	3.7	4.5	4.4	2.5
20	H.sapiens (xscad) mRNA, 3405p; nt_non_genomic(identity):40_TCC_13F11_M13F.fa	g|533988|emb|Z38852.1|HSXSCAD	TCC-13F_11	-1.2	-1.1	-2	1.6	1.6	1	-1.6	-1.3	-1	-1.5	1.6	2.1	2.2	1.2	1.6

TABLE I CONTINUED

	TABLE III			Low	G3	High	G3	G1/2	G2	G2						Low	G2/3	G2	G2	G2	G2					
normal	normal	normal	normal	T1	T1+TIS	T1	T1	Ts	Ts/T1	Ta	INV	normal	normal	normal	normal	Ta	T1	To	Ta	T1	Ta	INV	normal	normal	normal	
TC21A	TC22A	TC23A	TC24A	TC25A	TC28A	TC29A	TC30A	TC31A	TC32A	TC33A	TC34A	TC35A	TC36A	TC37A	TC38A	TC38A	TC40A	TC41A	TC42A	TC43A	TC44A	TC45A	TC46A	TC47A	TC48A	secreted
-1.3	1	1.3	-2.1	2.6	2.4	2.2	2	2.1	2.3	2.4	-1	-3.9	-1.4	-1.8	-1.4	3.2	3.1	2.2	2.6	1.7	3.8	1.1	-1	1	-1.1	3.9
-1.2	1	1.2	-1.9	2.1	2.2	2	1.8	1.8	2	1.9	-1.2	-4.5	-1.6	-2.3	-1.6	2.9	2.5	1.8	2.4	1.8	3.6	1.1	-1.1	1	-1.1	3.8
-1.6	1	1.8	-1.7	4	1.5	3.5	3.6	4.3	3.2	2.1	1.1	-3.3	-1.2	-1.4	-1.3	3.5	-1.8	2.4	2.5	1.8	5.6	-1.3	-1.1	1	-1.2	3.8
-1.5	1	1.7	-1.8	4.4	-1.3	2	2.1	1.6	4.8	2	3.3	-1.4	-1	-1.8	-1	4.1	2.3	1.7	1.6	2.2	3.3	1.2	1.1	1	-1.1	-1.8
1.3	1	1.9	-1.1	4.4	-1.1	3.6	3.2	3.7	6	2.8	1.8	-4	1	-1.7	-1.3	3.4	4.5	1.3	3.3	2.3	3.4	-1.6	-1.2	1	-1	-1.2
-1.3	1	1.4	-1.1	2.7	1.6	1.2	1.3	4.3	3.7	2.2	1.1	1.1	-1	-1	1	2.3	3.4	2	1.9	2.2	3.1	1.1	-1	1	-1.1	1
-1.1	1	1.9	-1.1	3.5	1.2	2.4	2	2.4	3.1	1.7	1.8	1.2	1	-1.6	-1	2.4	2.7	1.1	2.4	1.5	2.9	-1.4	1.1	1	-1	-1.4
1.2	1	1.5	1.7	2.4	3.9	2.2	1.7	2.5	2.6	2.7	2.1	2	-1.3	-1.8	1	1.4	1.6	2.6	1.6	2	3	1.2	-1.1	1	-1.3	-2.2
1.1	1	1.4	1	3.7	7	2	-1.1	2.5	1.8	2.5	1.3	-1.3	1.1	1.2	1	1.8	4	1.7	2.5	1.4	2.3	1.6	1.3	1	1.1	-2.8
-1.2	1	1.2	-1.2	2.4	-1.1	2	2.7	13	1.8	3.8	3	1.4	1.1	1.4	1.1	3.8	3.4	3.2	1.4	2.6	3.8	2.7	1.1	1	1	
-1.8	1	1.2	-2.4	3.8	-1.1	2.4	1.1	2.3	2.4	1.6	2.5	-1.6	-1.2	-1.8	-1.1	2.7	1.2	2.1	1.9	1.2	3.1	1.3	1.1	1	-1.3	-1.8
-2.5	1	1.1	1	1.8	-1.6	1	6.7	3.5	1	3.9	-1.1	-4.1	-1.6	-1.9	-1.1	1.1	2.8	2.5	2	2.2	3.8	2.1	1.1	1	-1.2	-1.2
1.1	1	1.4	-1.2	3.1	-1.3	1.2	1.1	3.1	1.6	1.8	-1.1	2	-1.2	-1.4	-1	2.3	2	1.8	1.9	1.3	2.8	1.4	1	1	-1.2	4.9
-1.6	1	1.2	-2	2.4	1.1	1.7	1.3	-1	2	1.9	-1.1	-2.3	-1.4	-1.6	-1.3	1.6	2.6	1.1	1.3	1.3	1.4	-1.3	1.1	1	-1.1	-1.5
-1.4	1	1.2	1.4	3.1	-1.3	1.8	1.2	2.3	6.7	-1.3	1.3	-1.3	1.2	1.3	1.1	1.9	1.5	1.9	2.5	1.8	2.3	1.1	-1.4	1	-1.2	
-2.1	1	1.1	-2.3	3.9	-1.3	2.5	2	3.7	2.8	2.5	-2.1	-1.2	1.1	-1.2	1.1	1.3	1.6	2	1.4	2	1.3	1.4	1.6	1	-1.1	3.6
-1.3	1	1.1	1.6	-1.3	6.7	6.7	1.9	1.8	1	1.7	3.5	1.5	1.3	1.2	1.1	1.9	2.8	2.4	1.7	1.4	1	2.5	1	1	1	1.9
1.3	1	2.1	1.4	3.8	-2.1	-1.6	1.5	5.3	-1.9	3.2	-1.8	-1.8	-1.2	-1.1	-1.1	2.2	1.6	2.3	2.8	2.8	3.1	1.1	-1.2	1	-1.2	-1.7
-1.3	1	1.6	1.2	3.7	-1.5	1.5	-1	2.1	6	-1.6	1.1	-1.3	-1.1	1	1	1.6	1.2	1.9	2.3	1.9	2.9	1.1	-1.3	1	-1.1	-1.8
-1.3	1	-1.1	-1.3	1.8	4.6	2.8	1.9	3	3.1	3.1	2.8	2.4	-1.1	-1.7	1.2	1.7	1.9	2.9	2	2.1	3.2	1.3	1	1	-1.2	-2.2

TABLE 2

					G2/3	G2/3	G2/3	G1/2						G2	G2	High	W
				T1	T1	T1	T1	T1	normal	normal	normal	normal	normal	T1	Ta	Ta	T1
	GeneDescription and clone ID	Accession	GeneID	TC2A	TC3A	TC4A	TC5A	TC6A	TC7A	TC8A	TC9A	TC10A	TC11A	TC16A	TC17A	TC18A	TC19A
1	Homo sapiens full length insert cDNA clone ZC48G12 ; nt_non_genomic(identity):03_TCC_57E11_T7.TXT.fa	gi|3493565|gb|AF0662 10.1|HUMZC48G12	TCC-57E_11	3.3	2.8	3.2	1.3	2.3	1	-1.3	-1.5	1.2	-1.2	2.2	2.6	3.7	1.9
2	xq01h07.x1 Soares_NHCeC_cervical_tumor Homo sapien ; est(identity):24_TCC_99C7_M13F.TXT	gi|7154678|gb|AW5186 98.1|AW518598	TCC-99C_7	2.8	4.3	5.8	6.9	-1.4	1	-1.3	-1	2.7	1.2	4	7.5	2.2	2
3	hh68c04.x1 NCI_CGAP_GU1 Homo sapiens cDNA clone IM ; est(identity):26_TCC_13H10_M13F_B04_032.ab1.fa	gi|7318401|gb|AW6132 16.1|AW613216	TCC-13H_10	2.2	1.8	2.1	1	1.2	1	-1.4	1.1	1	-1	1.3	1.2	1.3	-1.1
4	none:21_TCC_43E2_M13F.TXT		TCC-43E_2	2.7	4.7	7.3	1.5	1.7	1	2.3	-2.3	2.1	1.7	1.8	5.1	6.2	2.6
5	RC3-BN0063-170200-011-e12 BN0063 Homo sapiens cDNA ; est(identity):28_TCC_16D12_M13F_D04_041.ab1.fa	gi|8257938|gb|AW9977 04.1|AW997704	TCC-16D_12	2.7	5.9	2.4	6.6	-1.4	1	2.7	1.3	1.3	-1.2	3.6	4.4	-1.1	2.3
6	none:08_TCC_70E7_M13F_H01_015.ab1.TXT		TCC-70E_7	3.8	2.1	3.8	1.8	1.5	1	-1.8	-2.1	1.1	-1.1	3.5	4.8	2.9	2.6
7	Homo sapiens clone RP4-564D14, complete sequence ; nt_non_genomic(identity):14_TCC_9B6_M13F_F02_026.ab1.fa	gi|8468933|gb|AC0055 86.2|AC005586	TCC-9B_8	11	-1	3.9	4.1	-1.8	1	-1.8	1	-1.2	-1.4	3	3.7	1.3	1.2
8	Homo sapiens CGI-81 protein (LOC51108), mRNA ; nt_non_genomic(identity):11_TCC_101E11_M13F.TXT.fa	gi|7705788|ref|NM_016 025.1|	TCC-101E_11	6.1	2.1	3.1	1.6	1.3	1	-1.5	-1.4	1.1	1.2	2.8	7.2	4.4	2.1
9	Homo sapiens mRNA for KIAA0560 protein, complete ; nt_non_genomic(identity):72_TCC_37E11_M13F_H09_079.ab1.fa	gi|3327133|db|AB0145 60.1|AB014560	TCC-37E_11	8	-1.5	3.1	3.1	-1.3	1	-1	-1.2	-1.4	-1.8	1.8	2	-1	-1.3
10	H.sapiens GalNAc-T1 gene, 3'UTR ; nt_non_genomic(identity):54_TCC_30E5_M13F_F07_062.ab1.fa	gi|2292903|emb|Y1034 3.1|HSY10343	TCC-30E_5	3.3	2.3	-1.2	1.3	2	1	1.5	1.1	1.8	2.1	-1.1	1.7	1.7	1.2
11	none:15_TCC_71H8_M13F_G02_019.ab1.TXT		TCC-71H_8	1.7	1.4	-1	2.5	2	1	-1.5	-1.2	1.1	-1.3	2.1	2.8	3	1.6
12	Homo sapiens ETAA16 protein (ETAA16), mRNA ; nt_non_genomic(identity):09_TCC_101C11_M13F.TXT.fa	gi|9508580|ref|NM_019 002.1|	TCC-101C_11	6.1	2.2	3.2	1.9	1.2	1	-2.3	-1.4	-1	1	2.6	5.8	4.2	1.9
13	Homo sapiens cDNA FLJ10561 fis, clone NT2RP4001 51 ; nt_non_genomic(identity):24_TCC_12F3_M13F_H03_031.ab1.fa	gi|7022782|dbj|AK0017 23.1|AK001723	TCC-12F_3	1.4	2	1.1	-1.3	1.8	1	-1	1.1	2	1.2	1.2	1.7	1.5	1.3
14	hh75c10.x1 NCI_CGAP_GU1 Homo sapiens cDNA clone IM ; est(identity):59_TCC_34D5_M13F_C08_065.ab1.fa	gi|7318786|gb|AW6136 10.1|AW613610	TCC-34D_5	1.8	2.5	1.8	1.1	1.4	1	1.1	1.1	1.9	1.4	-1.7	-1.2	-1.1	-1.5
15	none:15_TCC_67C3_M13F.TXT.fa		TCC-67C_3	3.5	2.8	2.9	1.3	2.3	1	-1.1	-1.6	1.2	1.2	1.6	2.6	2.9	1.7
16	yj81a11.r1 Soares breast 2NbHBst Homo sapiens cDNA ; est(identity):29_TCC_17A5_M13F_E04_034.ab1.fa	gi|643537|gb|R70320.1 |R70320	TCC-17A_5	2.7	2.6	1.9	1.7	9.3	1	1.2	1.5	1.8	1.6	1.5	-1	1.4	-1.2
17	hypothetical protein C50F7.2 - Caenorhabditis elag ; CONTIG_rv(strong):31_TCC_10E8_M13F.fa	gi|7497781|pir||T29298	TCC-10E_8	1.1	1.4	1.8	1.5	-1.1	1	1.3	1.3	1	-1.1	-1.1	-1.7	-1.7	-1.7
18	none:13_TCC_71E4_M13F_E02_018.ab1.TXT		TCC-71E_4	1.7	1.8	1.3	2.3	2.1	1	-1.4	-1.3	1	-1.1	1.8	2.3	2.7	1.8
19	Homo sapiens mRNA; cDNA DKFZp434L0310 (from clone ; nt_non_genomic(identity):57_TCC_6IC9_M13F_A08_056.ab1.TXT	gi|8808331|emb|AL137 591.1|HSM802346	TCC-6IC_8	3.7	2	3.2	1.7	1.5	1	-1.6	-1.3	1.1	-1.5	1.1	1.8	1.9	1.5
20	none:44_TCC_70E8_M13F.TXT.fa		TCC-70E_8	4.1	2.2	3.7	1.7	1.5	1	1.3	-1.7	1.1	-1	2.9	4.3	2.4	1.9

TABLE 2 CONTINUED

T1	normal	normal	normal	normal	Low T1	G3 T1+T1S	High T1	G3 T1	G1/2 Ta	G2 Ta/T1	G2 Ta	INV	normal	normal	normal	normal	Low Ta	G2/3 T1	G2 Ta	G2 Ta	G2 T1	G2 Ta	INV	normal	normal	normal	
TC20A	TC21A	TC22A	TC23A	TC24A	TC25A	TC26A	TC28A	TC30A	TC31A	TC32A	TC33A	TC34A	TC35A	TC36A	TC37A	TC38A	TC39A	TC40A	TC41A	TC42A	TC43A	TC44A	TC45A	TC46A	TC47A	TC48A	secrtd
1.5	1	1	1.7	-1.1	2.9	2.1	2	1.9	1	4.4	2.5	2.3	2.3	1.9	1.9	1.3	2.3	1.9	1.9	2.1	2.3	2.3	2.4	1	1	-1.1	-1.9
1.2	1.2	1	1.9	1.4	2	1.3	-1.1	3.2	1.1	9.1	-1.6	1.6	1.9	1.2	1.7	1.1	3	3.7	2.4	2.2	4.6	3.5	1.2	-1.2	1	-1.1	
-1.1	-2	1	-1	-1	-1.1	3.4	2.9	2.7	3.1	6.5	3.7	3.2	2.3	2.5	1.6	2.9	2.8	3.7	3.7	3.3	3.4	3.6	3.4	1.7	1	3.6	1.9
1.1	-1.1	1	1.6	-1.1	3.2	-1.4	2.6	2.3	2.7	3.5	2	1.4	-3.6	-1.2	-2.3	-1.6	2.4	3.2	1.2	2.1	1.9	3.2	-1.6	-1.4	1	-1.1	-1.3
-1.3	-1.2	1	1.1	2	1.3	-1	2.1	3	2	2	-2.2	1.1	1.2	-1.2	1.3	-1.2	6.6	1.3	1	2.3	5	2	1.1	-1.2	1	1.1	
2.3	-1.4	1	1.6	-1.6	3.6	-1.2	2.2	1.1	2.2	2.3	1.7	2.4	-1.6	-1.2	-2	-1.1	2.6	1.1	1.9	1.9	1.2	3.2	1.3	1	1	-1.3	-1.6
1	1.6	1	1.5	2.2	1.1	1.6	12	3	4.2	2.5	-1.5	9.8	1.1	1.2	1.2	-1.1	6	4.3	1.5	1.3	7	11	1.3	-1	1	1.1	1.3
2.1	-1.4	1	1.4	-1.6	2.4	-1.2	1.7	1.6	1.4	3.9	1.6	3	1.2	1.1	-1.3	-1	3	2.1	1.6	1.4	1.7	3.1	1.2	-1.1	1	-1.2	1.9
-1.2	1.2	1	1.1	1.4	-1.3	1	7.1	2.3	3.2	2.4	-1.2	6.1	1.2	1.5	1.2	1.2	4.7	4.5	-1.1	1.1	4.7	6.7	1.1	1.1	1	-1	1.6
1.3	1.3	1	1.2	1.6	1.5	1.4	2.5	2.3	6.1	3.6	1.5	2.1	1.4	1.6	1.7	1.4	2.2	1.3	2.6	2.2	2.7	2	1.7	1	1	1.1	-2.2
1.1	1	1	1.2	-1.1	2.2	3.5	2	1.6	2.4	2.7	2.4	1.9	1.6	-1.4	-1.9	1	1.3	1.6	2.9	1.6	1.9	2.6	1.2	-1	1	-1.2	-2.1
1.9	-1.1	1	1.4	-1.3	2.3	-1.1	1.5	1.6	1.4	3.7	1.7	2.6	1	1.1	-1.1	-1.1	3.1	2	1.5	1.6	1.6	2.5	1.2	-1.2	1	-1.3	-1.6
-1	1.1	1	1.2	1.2	1.6	1.6	2.6	4.1	5.5	2	2.4	2.2	3.1	1.9	1.6	1.6	2.5	1.6	1.7	2.1	2	2.1	2	1	1	1.1	-1.2
-1.6	-1.6	1	-1.2	1.4	-1.3	2.1	1.6	2.4	2.3	3.5	2	1.9	1.7	1.6	1.7	1.6	3	2.9	2.6	2.1	2.4	2.6	1.5	1.1	1	1.4	
1.3	-1	1	1.3	-1.1	2.3	1.9	1.5	1.9	-1.4	3.2	2	2	1.9	1.3	1.6	1	1.7	1.6	1.4	1.6	1.6	2	2	-1.1	1	-1.3	-1.6
-1.1	1.4	1	1.4	1.6	-1.2	1.1	-1	3	12	2.2	3.4	1.3	1.5	1.3	1.9	1.3	2.6	1.9	3.1	1.4	2.6	2.6	1.3	1	1	-1	
-1.1	1.5	1	-1.2	1.5	-2	3.6	2.2	2.4	3.2	4.4	3.6	2.2	3.2	-1.1	1	1.1	1.6	2.7	3.1	2.3	1.6	1.4	2	1	1	1.1	
2.4	-1.4	1	-1.1	-1.4	1.4	3.5	1.9	1.6	2.2	2.3	2.2	1.6	1.7	-1.9	-1.7	-1	1.4	1.6	2.1	1.6	1.6	2.9	1.2	-1.1	1	-1.2	-2.3
1.6	-1.6	1	1	-1.4	1.6	1.6	1.3	2.2	2.4	1.3	1.4	1.6	1.6	1.3	2.3	1.2	3.3	2.6	2.5	1.9	1.4	2.3	1.6	-1.1	1	-1.6	
2.9	1.6	1	1.3	-1.9	3.2	-1.3	1.6	-1.2	1.7	1.6	1.2	1.9	-1.7	-1.4	-2.1	-1.4	1.9	-1	1.5	1.3	1	2.6	1.3	-1.1	1	-1.9	-1.9

TABLE III

>40_TCC_13F11_M13F.fa TIME: Sun Sep 10 11:42:06 2000 trimming information: raw_sequence:582 (high quality:29-320) sequence:97-252 [length:156]
TCCGTCTCATTGAGGGTCCTGAGGAAGTTGATCTCATCATTCAGGGCATC
CACCTTGGCCTCCAGCTCCACCTTGCTCATGTAGGCAGCATCCACATCCT
TCTTCAGCACCACAAACTCATTCTCAGCAGCTGTGCGGCGGTTAATTTCA
TCTTCG

>04_TCC_94G3_M13F.TXT.fa ,constant: 15, poly A: yes
AAGGCTTATTCCATCCGGACCGCATCCGCCAGTCGCAGGAGTGCCCGCGACTGAGCCGCC
TCCCACCACTCCACTCCTCCAGCCACCACCCACAATCACAAGAAGATTCCCACCCCTGCC
TCCCATGCCTGGTCCCAAGACAGTGAGACAGTCTGGAAAGTGATGTCAGAATAGCTTCCA
ATAAAGCAGCCTCATTCTGAGGCCTGAGTGAAAAAAAAA

>20_TCC_60H4_M13F.TXT.fa ,constant: -1, poly A: no
CANTATATAACNAATTGGAGCTCAATNGCNCGCGGNCGCGTGTCTTCTGGGTAGAGGGAT
GNGAAGGAAGGGACCCTTACCCCCGGCTCTTCTCCTGACCTGCCAATAAAAATTTATGGT
CCAAGGNAAAANA

>26_TCC_44C1_M13F.TXT.fa ,constant: -1, poly A: no
ACTCATTGAACTTGAGCTCCGANTCCTGATTCNCATCNAAGCTCTNNATCTGCTCATCAN
GAGANCCCACATCCTTGAGCAGATGGNGCANCTGCTGNTTAACCANCTCTNNGAACTCGN
AGANNNTAAGGCTATCCTTCCGGNCCTCCTGCCTTGCAAAGGTGAAGAAAGTGGTGNNCA
CNGTCNCAATGGANTCCTCTAGCTCTGTCAGTGGTTCTGCTGCNATTATGGAACCTGAGG
CCAAAGCTGATGTCCTCAAGGGGCTAGCTGACCTTTGTCAGGGCTGACCTCTCCTCAGCG
GCAGCAGGGCAGAGTGCTGAACCCAGGAACCCACAGATCCTCCCCGNTCCTGTCTCCCGG
TGACAAGGGTCCTGGAACGGGGCGTCTCTGACTCCCTGCTCCAGGACGGGTTTAAGT

>29_TCC_48G1_M13F.TXT.fa ,constant: -1, poly A: no
ACTTTGAGAAGGCAGGACTCAAATGATGCCCTGGAGATGTCACAGATTCCTGGCAGAGCC
ATGGTCCCAGGCTTCCCAAAAGTGTTTGTTGGCAATTATTCCCCTAGGCTGAGCCTGCTC
ATGT

>31_TCC_65B9_M13F.TXT.fa ,constant: -1, poly A: yes
GACTAGAACCCACCCCCTTNCCTTCCAGCCTTTCTGTCATCATCTCCACAGNCCANCCAT
CCCCTGAGCACACTAACCATCTCATGCAGGCCCCACCTGCCAATAGTAATAAAGCAATGT
CACTTTGTTAAAACATGAAAAAAAAA

>47_TCC_91B11_M13F.TXT.fa ,constant: -1, poly A: yes
CTAGTATACACTCCNCATAGNATACGTTGCAGCTCAATTGCGCGCGGNCGCGGACGACGA
CCTGCGAGGGTGTCTTCTGGGTAGAGGGATGGGAAGGAAGGGACCCTTACCCCCGGCTCT
TCTCCTGACCTGCCAATAAAAATTTATGGTCCAAGGAAAAAAAAA

>10_TCC_53H11_T3
TTTTTTNNATNTTATTTTGGGTATTGGTGTTNTTTCTTTTTTCCTCTTNCCTTCTTAACT
CAAGACTTGTAGTGTTGTAAACCTGCCTCACAAAATACATGGTAATAACTTNTCTTTAAA
AAAANAAAAAAGACAGNCTTNACACCATTTCTAATNGNANNACTATTTTTGGGCAATGTT
ATGCACCACTTCAATTTCCCCATTGTGACCCCTATCACTTCATTTGATATCCCTTTTNGA
CCCANCCATCTCCTTCATATATGGGCATGTCCATAGATTGACAAAGAAAGTTTACACTTT
NGAATAAAGATGCAAAGTATGCAAAAACATTAATACTGATGCNAAAAAAANTANAAAAA

>07_TCC_57B3_M13F.TXT ,constant: -1, poly A: yes
GGTACCGACGGACCTGCGGAGACTCCTGCCCTGTTGTGTATAGATGCAAGATATTTATAT
ATATTTTTGGTTGCAATATTAAATACAGACACTAAGTTATAGTATATCTGGCAAGCCAAC
TTGTAAATCACCACCTCACTCCTGTACTTACCTAAACAGATATAAATGGCTGGTTTTTAA
GAAAAAAAAA

>11_TCC_25F2_M13F.TXT ,constant: -1, poly A: no
ACCCTGGGAGAGAAGTTTGAAGAAACCACAGCTGATGGCAGAAAAACTCAGACTGCTGCA
ACTTTACAGATGGTGCATTGNGTCAGCATAGGAGTGAGATGGGGAAGGAAAGCACANTAA
CAAGAAAATTGANAGATGNTAAATTAGTGNTGGAGTGTGTCATGAACAATGCACCTGT

>25_TCC_50G5_M13F.TXT ,constant: 17, poly A: yes
TAGTGTGGAAGCATAGTGAACACACTGATTAGGTTATGGTTTAATGTTACAACAACTATT
TTTTAAGAAAAACATGTTTTAGAAATTTGGTTTCAAGTGACATGTGTGAAAACAATATCG
ATACTACCATAGTGAGCCATGATTTTCTAAAAAAAAA

>26_TCC_50G6_M13F.TXT ,constant: 17, poly A: yes
TAGTGTGGAAGCATAGTGAACACACTGATTAGGTTATGGTTTAATGTTACAACAACTATT
TTTTAAGAAAAACAAGTTTTAGAAATTTGGTTCAAGTGACATGTGTGAAAACAATATTGT
ATACTACCATAGTGAGCCATGATTTTCTAAAAAAAAA

>26_TCC_75E3_M13F_B04_032.ab1.TXT ,constant: 16, poly A: yes
AAAGAGGGCGGCAGGGGCCTGGAGATCCTCCTGCAGACCACGCCCGTCCTGCCTGTGGCG
CCGTCTCCAGGGGCTGCTTCCTCCTGGAAATTGACGAGGGGTGTCTTGGGCAGAGCTGGC
TCTGAGCCGCCCTCCATCCAAGGCCAGGTTCTCCGTTAGCTCCTGTGGCCCCACCCTGGG
CCCTGGGCTGGAATCAGGAATATTTTCCAAAGAGTGATAGTCTTTTTGCTTTTTGGCAAA
ACTCTACTTAATCCAATGGGTTTTTCTCTGTACAGTAGATTTTCCAAATGTAATAAACTT
TAATATAAAGTAAAAAAAAA

>30_TCC_76B3_M13F_F04_042.ab1.TXT ,constant: 16, poly A: yes
AAAGTCATCCTCCGTCTACCAGAGCGTGCACTTGTGATCCTAAAATAAGCTTCATCTCCG
GGCTGTGCCCCTTGGGGTGGAAGGGGCAGGATTCTGCAGCTGCTTTTGCATTTCTCTTCC
TAAATTTCATTGTGTTGATTTCTTTCCTTCCCAATAGGTGATCTTAATTACTTTCAGAAT
ATTTTCAAAATAGATATATTTTTAAAATCCTTAAAAAAAAA

>38_TCC_56E11_M13F.TXT ,constant: -1, poly A: yes
CTCTCCAGTTTGCACCTGTCCCCACCCTCCACTCAGCTGTCCTGCAGCAAACACTCCACC
CTCCACCTTCCATTTTCCCCCACTACTGCAGCACCTCCAGGCCTGTTGCTATAGAGCCTA
CCTGATGTCAATAAACAACAGCTGAAGCAAAAAAAAA

>46_TCC_78B11_M13F_F06_058.ab1.TXT ,constant: 16, poly A: yes
AGGAAAGGTGNGNGCTGGAAGCACTGAACCTACCTCATCCTCCTGGTGGGTGTGGCTACC
CTCGCCACCCCAAATTCCATGTCATTAAAGAACAGCTAAATTCAAAAAAAAA

>53_TCC_79G2_M13F_E07_054.ab1.TXT ,constant: 16, poly A: no
TGTCCGTCTTCACCCATCCCCAAGCCTACTAGAGCAAGAAACCAGTTGTAATATAAAATG
CACTGCCCTACTGTTGGTATGACTACCGTTACCTACTGTTGTCATTGTTATTACAGCTAT
GGCCACTATTATTAAAGAGCTGTGTAACATCAAAAAAA

>82_TCC_89G3_M13F_B11_092.ab1.TXT ,constant: 16, poly A: yes
CAGGAGACCATCCGCGTCACCAAGCCCTGCACCCCCAAGACCAAAGCAAAGGCCAAAGCC
AAGAAAGGGAAGGGAAAGGACTAGACGCCAAGCCTGGATGCCAAGGAGCCCCTGGTGTCA
CATGGGGCCTGGCCCACGCCCTCCCTCTCCCAGGCCCGAGATGTGACCCACCAGTGCCTT
CTGTCTGCTCGTTAGCTTTAATCAATCATGCCCTGCCTTGTCCCTCTCACTCCCCAGCCC
CACCCCTAAGTGCCCAAAGTGGGGAGGGACAAGGGATTCTGGGAAGCTTGAGCCTCCCCC
AAAGCAATGTGAGTCCCAGAGCCCGCTTTTGTTCTTCCCCACAATTCCATTACTAAGAAA
CACATCAAATAAACTGACTTTTTCCCCCCAAAAAAAAA

>35_TCC_21D6_M13F_C05_037.ab1.fa TIME: Wed Aug 9 12:48:31 2000
trimming information: raw_sequence:889 (high quality:34-340)
sequence:95-456 [length:362]
CTTTGACGTGGAGAGGAACTCCTGCAATAACTTCATCTATGGAGGCTGCC
GGGGCAATAAGAACAGCTACCGCTCTGAGGAGGCCTGCATGCTCCGCTGC
TTCCGCCAGCAGGAGAATCCTCCCCTGCCCCTTGGCTCAAAGGTGGTGCT
TCTGGCGGGGCTGTTCGTGATGGTGTTGATCCTCTTCCTGGGAGCCTCCA
TGGTCTACCTGATCCGGGTGGCACGGAGGAACCAGGAGCGTGCCCTGCGC
ACCGTCTGGAGCTCCGNAGATGACAAGGAGCAGCTGGTGAAGAACACATA
TGTCCTGTGACCGCCCTGTCGCCAAGAGGACTGGNGAAAGGGAGGGGAGA
CTATGTGTGAGC

>46_TCC_27H5_M13F_F06_058.ab1.fa TIME: Wed Aug 9 12:48:35 2000
trimming information: raw_sequence:892 (high quality:169-406)
sequence:170-287 [length:118]
AAAAAGAGTAAAACACTTTCAGTTTCTCCCCTTTAGCCCCTAAAACAACA
TCTTACAGTCTGGATCTGGATCTACCTATACAGTCCTACATTAGCTTCTA
AAATATTTGTCAGGAGGG

TABLE IV

>31_TCC_10E8_M13F.fa TIME: Sun Sep 10 11:42:01 2000 trimming information: raw_sequence:549 (high quality:25-313) sequence:98-313 [length:216]
CCCAAATGGAATGTTGCCCCCTTAAACACCATTTTCCCTCCAGGACCACC
TTGGTTTCTAGGCACTGTGGTTCTTGGCAGGGGCTGTCTTAGGTAAAAGG
GTAGTTGTGGAGCTACAGTCTGAAGAACATAGCTTGGGCTCAAGTTCAAA
TGAGCCATCTTTTTCCTTTGCGTTTTTCTTGACTGAAGGTGAGATGTTAT
TTGTGGCATGTGAACT

>09_TCC_101C11_M13F.TXT.fa ,constant: 16, poly A: yes
ACAAAGACTGCTGATAACTATCTGTGATTGATAGGAAATTTTTTTTCTTGATTTCTCTGT
GAGAAATGTAATGCTGACTTTTATAAAGCCTGGACTTCTACTTTATTTAATAAATCAATG
TTTGCAATGGTAAAAAAAAA

>11_TCC_101E11_M13F.TXT.fa ,constant: 15, poly A: yes
GCAATAAAGCTGTCCATTCAATTCCAAATACTGGTTTTAAGNGTATAGCCACTGATATTC
TTTCATGTNTAGAAATTCTTTCTGTTATTATTCAAGAAAATGTTTTTAATCATGCTAATA
AACTTTTTTGGAGATGAAAAAAAAA

>15_TCC_57C3_M13F.TXT.fa ,constant: -1, poly A: no
GGNACCACGTACCTGCTGAATGTNTCNNCGNNATGNCGNCAGGCCATGCTGTTGCTGATN
TANTACTNTGAAAATANGGATATCATGATGGGAATGCATGTCATGAGGTCCAGANTCGTT
CTACTGTCNATAANCTGTNTACTNGCGTTGANAANAAANGATGTCAAAGNCCCCCCGTAA
AAANGTA

>44_TCC_70E8_M13F.TXT.fa ,constant: 15, poly A: yes
GTCCCAGTCTTCACCAGGTGTCTCTCCTCTTTTACTCAGGAGGACTTTCCCAGGAAAACC
ATGCCACTAGCAAAAAAAAA

>03_TCC_57E11_T7.TXT.fa ,constant: 16, poly A: yes
TGAGTGTCTTCAGGCCAACCTGGTGGAAATGTTGTTCTCTGAAGATTAAGATTTTAGGAT
GGCAATCATGTCTTGATGTCCTGATTTGTTCTAGTATCAATAAACTGTATACTTGCTTTG
AATTCATGTTAGCAATAAATGATGTTAAAAAAAAA

>08_TCC_70E7_M13F_H01_015.ab1.TXT ,constant: -1, poly A: no
GGATCGACGACCTGCTTCCCAGANGCGNNCNNGAGGNCCNCTTGTTNNNGNCNNGNANAC
NNACCCANTTNANTTNNAGCCTTTNTGNAATAAATATACACAGGCCACCCATGCCNTGAG
CACACTAACCACNTGATGCAGGCCCCACCTTGCCAATAGTAATAAAGCANTGGGACGTTT
TTTA

>13_TCC_71E4_M13F_E02_018.ab1.TXT ,constant: -1, poly A: no
GGGCCAAAGCCCGNGCATCCAANCCCANGCAAGGNACAAANGANCNNGGAGAGGANNACC
CAAGCANNTNNCAACCATCAAATGGAGGGCANGCCCGGGG

>15_TCC_71H8_M13F_G02_019.ab1.TXT ,constant: -1, poly A: no
GGGCCAAAGCCGNGCATCCAANCCCANCGCANGGNANAAANGANGANGGANANGGATNAC
CCANGCCTNTATTAACCATCAANTGGGANGGCAAGCCCGGGGCATNTATTGATT

>21_TCC_43E2_M13F.TXT ,constant: -1, poly A: no
AGGACCCCTGAANACNACACAGATCTGTGNGAAACAANGGNACNTAGCGTCCCNAAAGTG
CCNGGTTNNNGTANNCNNAGNGNGNGACCNGNGCNCATNT

>24_TCC_96C7_M13F.TXT ,constant: 16, poly A: yes
ATCCAGAGACCATCAATCCTGCTAGAGTGCAGGGTGGCAAGCACCCAAGGGTGGCTGACC
AAGACTGCAGAGTCTCCTCCATCTTCAGGTCCATTCAGCCTCCTGGCATTTAACTACCAG
CATCCAGTGGTCCCCAAGGAATCCCTTCCTAGCCTCCTGACATGAGTCTGCTGGAAAGAG
CATCCAAACAAACAAGTAATAAATAAATAAATAAACTCAAAAAAAAA

>57_TCC_80C9_M13F_A08_056.ab1.TXT ,constant: 17, poly A: yes
CTGCAGGAGTCAGCGTTCAATCTTGACCTTGAAGATGGGAAGGATGTTCTTTTTACGTAC
CAATTCTTTTGTCTTTTGATATTAAAAAGAAGTACATGTTCATTGTAGAGAATTTGGAAA
CTGTAGAAGAGAATCAAGAAGAAAAATAAAAATCAGCTGTTGTAATCACCTAGCAAAAAA
AAA

>14_TCC_9B6_M13F_F02_026.ab1.fa TIME: Wed Aug 9 12:48:25 2000
trimming information: raw_sequence:871 (high quality:73-413)
sequence:98-394 [length:297]
CACGCATATGGGGCCAGTTCCACATATTTGGCAACCAGACCAGCATCCAG
GACAACACAAAGTATGTTGTTTGTTGTTAGAGGGCTTGGGACATTTCACT
CTTTGCCAGCCTCAGCTTAATCCAGGAGACAAAGATTATTTTCCTTATTA
TCTCTTCTGCATAGGATCTGCAATCAGAACTATTGAACTTCTCCATTCAG
ACCGCCACTCACACCTATGGGAAAAGGGTAATGTATCATCGGCTTAGCAA
CAGGGAATACTATTCGTATGATGGAAAATGGGGACAAAAGGCTTTGG

>24_TCC_12F3_M13F_H03_031.ab1.fa TIME: Wed Aug 9 12:48:28 2000
trimming information: raw_sequence:842 (high quality:82-340)
sequence:98-476 [length:379]
CTATGAATAGCTTCTTGCTTTATGACTTTAGGATTAACTTGTAAAAAACA
TATCCTGAACTAAGATATGCAAAATACTCATTTTCAAGTTATGGAAATGT
GTTTGTGGCATATAGGACTGTGGGGTCTGTGTGTGTAGTGAGAGTGTGTA
TCCACTATTATAACTGGAATTTAATTTACATTCATAAACTACTATATTTC
CCATCTTGCAAATCATTTTATGTCTCATCTGTTTTTCCTTTCGGNTATAT
CTTTGGNTTTGAATACCAACATTTAAAATGATGGNATTTTATCTTTTAAA
CTTAAAAATTATTTAATACAGCTATATGGACCTTATAAAATTGATTTCTT
ATTTATTATTAGACATTACTACTAAAAGG

>26_TCC_13H10_M13F_B04_032.ab1.fa TIME: Wed Aug 9 12:48:29 2000
trimming information: raw_sequence:874 (high quality:67-356)
sequence:99-261 [length:163]
CTAACCCACGATTCTGAGCCCTGAGTATGCCTGGACATTGATGCTAACAT
GACCATGCTTGGGATGTCTCTAGCTGGTCTGGGGATAGCTGGAGCACTTA
CTCAGGTGGCTGGTGAAATGACACCTACGAAGGAATGAGTGCTATAGAGA
GGAGAGAGGAGTG

>28_TCC_16D12_M13F_D04_041.ab1.fa TIME: Wed Aug 9 12:48:29 2000
trimming information: raw_sequence:866 (high quality:71-411)
sequence:95-602 [length:508]
CAGCTGATGTCATGTGGTGCTGAGAAGAAAGCAGATCACACTTCATCACA
GAAAGAATGCCTTGTGATTATCTTCTCCACATCTGAAATTCCTTTTGACA
CCTGCATTGGGCCGACTGCCATTCCCATGACTGCTGCACCTGCGTTTTTA
GAGAATGCCTCATAACCCACTGATTCTCATTCACAGAGAATGGGAATACG
GAATGAAGAAAGATTCCAGCAGCTTATAGAAGGATAGCAATATTTTGGGA
CAGGGAAAATCCTGTCATACCTCACCTCTTCCTCAGGAGGAGTTCTGAGC
TGGTCCTGCTTTTCATAGNTGTTTCTTTTCTTCCACTTAAGAACTCATAG
ATTTTTCTTACTGTCCTAAGGAAGTCCTTACCTCTGAGGTATCTCCTCAA
TGAATACTGTTTTCAAGGCTGAAATAGTTCATTATGTTAATAACCTTCTT
TATGTTCTCAGGGAAATGCTTAGGTGGTGTCACAAAAAGGGCCTTTTCTT
TNCTTTNC

>29_TCC_17A5_M13F_E04_034.ab1.fa TIME: Wed Aug 9 12:48:30 2000
trimming information: raw_sequence:861 (high quality:83-477)
sequence:99-187 [length:89]
CTTCAAAAAGTGTATTGTCAAACATACCTAACTTTCTTGCAATAAATGCA
AAAGAAACTGGAACTTGACAATTATAAATAGTAATAGTG

>54_TCC_30E5_M13F_F07_062.ab1.fa TIME: Wed Aug 9 12:48:37 2000
trimming information: raw_sequence:836 (high quality:65-394)
sequence:90-235 [length:146]
CAATTTGTTATAGTATAGTATCAAATTTCTATATAGATTTTATACCTCAG
TGGGGAAAAATAACTGATTCCAATGACATTCATTTTGTTTTCATCTGTGA
TAGTCATGGATGCTTTTATTTTCCTTGGGGTGCTGAAATTGAGCTG

>59_TCC_34D5_M13F_C08_065.ab1.fa TIME: Wed Aug 9 12:48:39 2000
trimming information: raw_sequence:875 (high quality:63-434)
sequence:96-244 [length:149]
CCTGCCAAAATCCTACCACAGGATAACATTACAAGCAAAAAATTTACATG
TTCCAAAGTCTACCACACTCAAGAAGTTACTAAGAACTCTTGCAGAATAA
AAGTCACCATTTTAGAAATGCAAACCCACTTCCAACCTTTGCACAGTCC

>72_TCC_37E11_M13F_H09_079.ab1.fa TIME: Wed Aug 9 12:48:43 2000
trimming information: raw_sequence:899 (high quality:35-432)
sequence:97-444 [length:348]
CATTTTTAGTGACATTTTAAAAGCAGTCAGATTCTATAAATGGCAAGTAA
GCCTGAAGTGAGGATACTGCAATTTTCGGAGAAAAGAACAGCAGCTCTTT
AAGTGTTTGCATTTTCTATTTGGGGGGCAGGGAACTGTCATTCATTTTGC
ACAATTCTTGAACTGATGTCAGCACCCGAGTGGCTCCTGAATTTAAGTCT
GGGACGACATCTTTTATTTTTACATGAATCTTTAAACAATTCTGTGAGCA
AAGTTTGTAGCTGCTGGATTATTGTCTGTCTTTATAGCAAGTTCCAGTAA
ACCACAAGTATGGCAAAGCTTATCCAATTTTATGCTTGNAGCAGTCAG

CLAIMS

What is claimed is:

1. A method of diagnosing the presence of bladder cancer in a patient by analyzing a tissue sample from the patient for the presence of at least one expressed gene wherein the presence of the expressed gene is indicative of bladder cancer.

2. The method of claim 1, wherein the analyzing step further includes using mRNA from the expressed gene to hybridize to at least one of the sequences in Tables III or IV.

3. A polynucleotide sequence whose expression is indicative of bladder cancer.

4. The polynucleotide sequence according to claim 3, wherein said sequence is selected from the group consisting essentially of at least one of the sequences in Tables III or IV.

5. A marker for bladder cancer, wherein said marker is an expressed gene wherein the presence of the expressed gene is indicative of bladder cancer.

6. The marker according to claim 5, wherein said marker is a polynucleotide sequence which is selected from the group consisting essentially of at least one of the sequences in Tables III or IV.

7. A method of diagnosing bladder cancer by screening for the presence of at least one expressed gene wherein the presence of the expressed gene is indicative of bladder cancer.

8. The method of claim 7, wherein the analyzing step further includes using mRNA from the expressed gene to hybridize to at least one of the sequences in Tables III or IV.

9. Antibodies directed against the gene products of the sequences of claim 3.

10. The antibodies according to claim 9, wherein said antibodies are selected from the group consisting essentially of monoclonal, polyclonal and recombinant antibodies.

11. A method of treating bladder cancer-associated pathologies by administering to a patient a therapeutically effective amount of an antagonist of at least one protein as encoded by the nucleic acid sequences set forth in claim 3 or by the probes thereof.

12. A method of regulating bladder cancer-associated pathologies in a patient in need of such treatment by administering to a patient a therapeutically effective amount of at least one antisense oligonucleotide against the nucleic acid sequences set forth in claim 3 or dominant negative peptide directed against the sequences or proteins thereof.

13. A method of regulating bladder cancer-associated pathologies in a patient in need of such treatment by administering to a patient a therapeutically effective amount of at least one ribozyme against the nucleic acid sequences set forth in claim 3.

14. The method according to claim 13, wherein said administering step includes administering a ribozyme selected from the group consisting essentially of Group I introns, RNase P, hepatitis delta virus ribozyme, hammerhead

ribozymes and a hairpin ribozyme originally derived from the negative strand of the tobacco ringspot virus satellite RNA.

15. A gene therapy vehicle for delivering the sequences as set forth in claim 3 whereby the sequences are expressed in the target cells.

16. The gene therapy vehicle according to claim 15, wherein said vehicle is selected from the group consisting essentially of viral vectors, non-viral vectors, expression cassettes, and constructs.